Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica; pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide

EC Number: 272-697-1

CAS Number: 68909-20-6

CLH-O-0000006735-67-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

5 December 2019

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

International Chemical Identification:

Name: silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica; pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide

EC Number: 272-697-1

CAS Number: 68909-20-6

Index Number: -

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,1,1-TRIMETHYL-N-(TRIMETHYLSILYL)-, HYDROLYSIS PRODUCTS WITH SILICA; PYROGENIC, SYNTHETIC AMORPHOUS, NANO, SURFACE TREATED SILICON DIOXIDE

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1 IDENTITY OF THE SUBSTANCE

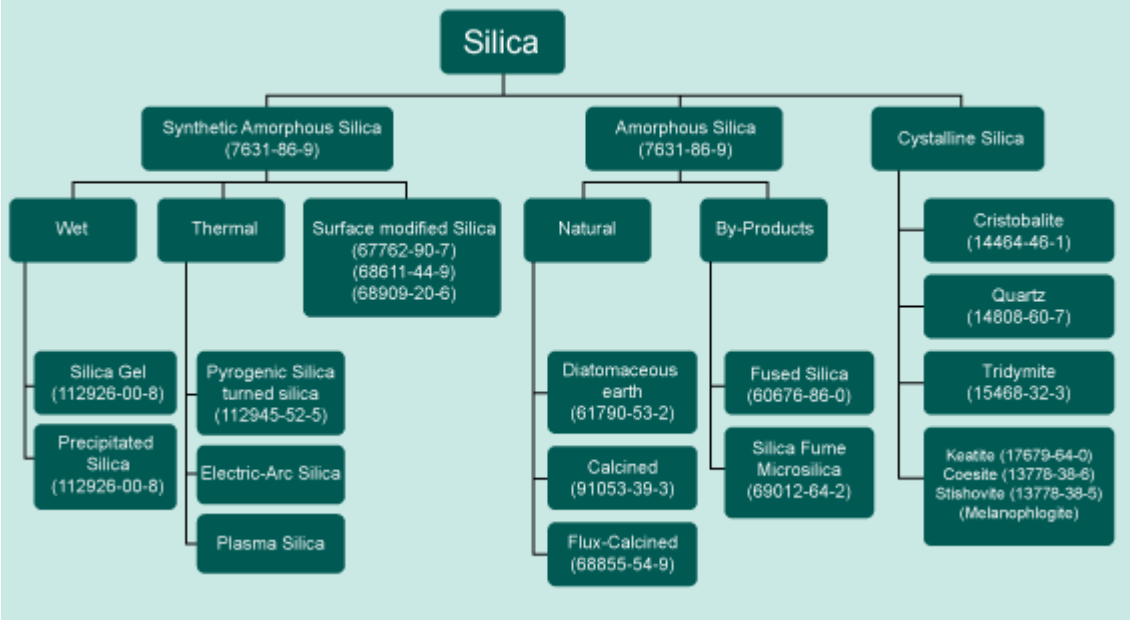
1.1 Name and other identifiers of the substance

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
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	See figure below figure 2
SMILES notation (if available)	Not relevant
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 SiO2 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 relevant

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 dossier: 6.9-8.6 nm
Shape of primary particles (TEM)	spherical

Figure 1: Polymorphs of silica covering crystalline as well as non-crystalline (amorphous) forms. CAS RN of the different forms are shown in square brakets. The specific surface-treated silica under this CLH proposal is derived from synthetic amorphous silica (SAS) (surface treated silica, CAS RN 68909-20-6).



Source: European Industrial Minerals Association

As shown in Table 1 above, the substance covered by this CLH proposal is "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) and a molecular formula as [SiO2]n-[OSi(CH3)3]m where n > m. The m corresponds to the surface treatment of silica with methyl (alkyl) groups. This description fit with two surface treated silica in this proposal: Aerosil R 812 and R 812 S.

The main difference between Aerosil R 812 and R 812 S is the density of superficial methyl groups which is slightly higher in Aerosil R 812 S (2-3% for R812 and 3.0-4.0 for R 812 S). The specific surface area (260 m²/g for R812 and 220 m²/g for R812S) is also a data which differentiates the Aerosils R 812 and R 812 S, but those two values remain in the same range. Aerosil R 812 and R 812S have been obtained by surface modification of the hydrophilic silica with 1,1,1-trimethyl-N-(trimethylsilyl)silanamine [CAS No. 999-97-3], that results in a trimethylsilyl-surface modified silica.

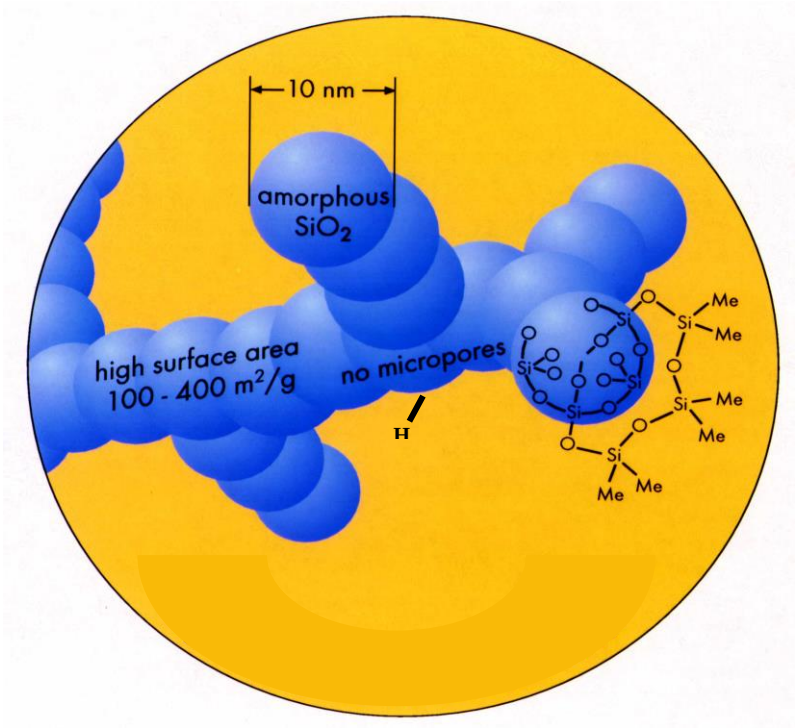
The surface modification of the hydrophilic silica with dichlorodimethylsilane [CAS No. 75-78-5] results in a dimethylsilyl-surface modified silica (Aerosil R 972, R 974, R 976) [CAS No. 68611-44-9], which are somewhat less hydrophobic than Aerosil R 812 S due to the lower density of superficial methyl groups.

Other surface treated silica are presented in this CLH proposal: Aerosil R 972, Aerosil R 974 and Aerosil R 976.

The difference between Aerosil R 972, Aerosil R 974 and Aerosil R 976, used in toxicology and ecotoxicology, and the Aerosils R 812 and R 812 S presented in this dossier is the molecule used for functionalisation of silica : Dichlorodimethylsilane for Aerosil R 972, Aerosil R 974 and Aerosil R 976 and 1,1,1-trimethyl-N-(trimethylsilyl)silanamine for Aerosils R 812 and R 812 S.

As a consequence, the density of superficial methyl groups in Aerosil R 972, Aerosil R 974 and Aerosil R 976 are slightly lower than the Aerosils R 812 and R 812 S .

Figure 2. Treated hydrophobic amorphous silica: here with dichlorodimethylsilane (HPV consortia 2003)



1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
Purity of silica	≥ 99.8 % (w/w) for pyrogenic (fumed)	none	Skin Irrit. 2 – H315 Eye Irrit. 2 – H319

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
	silica before and after the surface modification		Acute Tox 4 – H332 STOT RE 2 – H373

Table 3: 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 CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
Crystalline silica	<0.1%	None	STOT RE 1 – H372 (lung) (inhalation) STOT RE 2 – H373 Eye Irrit.2 – H319 Acute Tox 4 – H332	No

Crystalline silica is classified in group 1 (carcinogenic to humans) by IARC.

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
-					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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 Annex VI entry	No existing entry in Annex VI										
Dossier submitters proposal	To be determined	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		
Resulting Annex VI entry if agreed by RAC and COM	To be determined	silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica; pyrogenic, synthetic	272-697-1	68909-20-6	STOT RE 2	H373 (lungs; inhalation)	GHS08 Wng	H373 (lungs; inhalation)	EUH066		

		amorphous, nano, surface treated silicon dioxide									

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

Hazard class	**Reason for no classification**	**Within the scope of public consultation**
Explosives	data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Not relevant	No
Oxidising gases	Not relevant	No
Gases under pressure	Not relevant	No
Flammable liquids	Not relevant	No
Flammable solids	data conclusive but not sufficient for classification	Yes
Self-reactive substances	data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	Not relevant	No
Pyrophoric solids	data conclusive but not sufficient for classification	Yes
Self-heating substances	data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	data conclusive but not sufficient for classification	Yes
Oxidising liquids	Not relevant	No
Oxidising solids	data conclusive but not sufficient for classification	Yes
Organic peroxides	not relevant	No
Corrosive to metals	data conclusive but not sufficient for classification	Yes
Acute toxicity via oral route	data conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	data lacking	No
Acute toxicity via inhalation route	data conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	data lacking	No
Skin sensitisation	data conclusive but not sufficient for classification	Yes

Hazard class	Reason for no classification	Within the scope of public consultation
Germ cell mutagenicity	data conclusive but not sufficient for classification	Yes
Carcinogenicity	data conclusive but not sufficient for classification	Yes
Reproductive toxicity	data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	data lacking	No
Specific target organ toxicity-repeated exposure	A classification STOT RE 2 – H373 is proposed	Yes
Aspiration hazard	data lacking	No
Hazardous to the aquatic environment	Conclusive but not sufficient for classification	Yes
Hazardous to the ozone layer	data lacking	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

No previous or current classification is available for the Pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide.

RAC general comment

Synthetic amorphous silicas (SAS) are white, fluffy powders or milky-white dispersions of such powders (usually in water). SAS consists of nano-sized primary particles, of nano- or micrometre-sized aggregates and of agglomerates in the micrometre-size range. Hence, these materials fall under the general definition of engineered nanomaterials. SAS, including colloidal and surface treated forms, have been used extensively in medicinal/pharmaceutical, food and cosmetic products, but also in a wide variety of industrial applications including reinforcement and thickening agents in various systems such as elastomers, resins and inks. Consequently, the toxicological and ecotoxicological properties of the various forms of SAS have been studied and reviewed (Becker *et. al.,* 2013; Pölloth, 2012; EPA, 2011; ECETOC, 2006; OECD SIDS, 2004).

Under regulation (EU) 528/2012, "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), in particular in the control of fowl-infesting ectoparasites in poultry houses, by professional operators.

SAS are generally hydrophilic due the free silanol groups (Si-OH) on the surface of the particles. These silanol groups can be chemically derivatised by reacting with various agents to render the silica hydrophobic. There are many different methods of processing silica to become hydrophobic, mainly by adding hydrocarbon groups. Surface modification is usually done using organosilicon compounds. Surface modified (after-treated) SAS can be obtained either by physical or chemical reaction. The most common Si-organic compounds used for the treatment are hexamethyldisilazane (HMDS; CAS No 999-97-3), dimethyldichlorosilane (DDS; CAS No 75-78-5)

and polydimethylsiloxanes (PDMS; CAS No 9016-00-6). The first compound forms mono-functional moieties upon hydrolysis, whereas the latter two give rise to bi-functional units, as shown below.

- Hexamethyldisilazane (HMDS) → ≡Si–O–$Si(CH_3)_3$
- Dimethyldichlorosilane (DDS) → ≡Si–O–$[Si(CH_3)_2–O–]_{x = 1 - 3}$
- Polydimethylsiloxane (PDMS) → ≡Si–O–$[Si(CH_3)_2–O–]_{x = 3 – 6(10)}$

The substance covered by this CLH opinion belongs to the surface treated SAS with the chemical name "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), with a molecular formula of $[SiO_2]_n$-$[OSi(CH_3)_3]_m$, where n > m. The m corresponds to the surface treatment of silica with methyl (alkyl) groups. It is a synthetic amorphous silica (SAS), which has been modified with hexamethyldisilazane (HMDS, CAS 999-97-3) to give a hydrophobic SAS due to the trimethylsilyl-surface modified silica. In the present opinion, the specific silica will be referred to as "silanamine", "SAS-HMDS" or "silica silylate. The other non-surface treated silica, or crystalline silica substances are not within the scope of the CLH report, or the present opinion.

The DS included in the substance identity (SID) description the primary particle size, namely 6.9-8.6 nm, which is derived from the experimental data provided in the CAR by the applicant and covers specifically the products from this supplier. However, there are other major suppliers of similar products on the market, with product identifiers sharing the same CAS number, the same chemical name and similar primary particle size, with diameters in the range 5-20 nm (Pölloth, 2012).

RAC has included in the substance identity only the name and the EC and CAS numbers, 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)**. The name above includes both the EC name for the EC entry 272-697-1, and the common name of the biocidal active substance. Both parts are needed to define the entry. Since, in the name of the substance, the material is clearly defined as a "nanomaterial", RAC considers that there is no need to define the particle size, since all known commercial preparations of SAS-HMDS (5-20 nm) fall in the diameter range of a "nano" form.

Read-across between the different types of amorphous silica

The substance identified above to which this assessment applies, is a biocidal product, which is the result of the reaction of synthetic amorphous silica treated with hexamethylsilazane (HMDS), leading to a nano-form of silica characterised by CAS No 68909-20-6 and marketed under various trade names. An X-ray analysis showed that the substances to which this CLH assessment applies have a content of crystalline silica < 0.1%.

The surface modification of the hydrophilic silica with dichlorodimethylsilane [DDS, CAS No. 75-78-5] results in a dimethylsilyl-surface modified silica [Silica dimethyl silylate, CAS No. 68611-44-9], abbreviated SAS-DDS, which is somewhat less hydrophobic than SAS-HMDS due to the lower density of surface methyl groups. These substances are used as source substances in a read across assessment in the CLH report, as well as in this opinion, since they are structurally similar to silanamine and share physical, chemical and toxicological properties.

The surface modification of the hydrophilic silica with polydimethylsiloxane (PDMS, CAS # 9016-00-6) results in a dimethylsilyl-surface modified silica [Silica dimethicone silylate, CAS # 67762-90-7], abbreviated SAS-PDMS, which is somewhat less hydrophobic than SAS-HMDS due to the lower

density of surface methyl groups. These latter substances are also used as source substances in the read across assessment in this opinion, from studies found in the open literature and as supporting evidence to the key studies presented in the CLH report.

Characteristics such as chemical composition, particle size and shape, surface chemistry, surface area, solubility and rate of dissolution, hydrophobicity, zeta potential, dispersibility and dustiness all support the use of SAS-DDS and SAS-PDMS as read across substances for classification purposes with SAS-HMDS.

The DS has used the non-treated, hydrophilic SAS, in the read across for certain hazard endpoints in the CLH report. Although some physicochemical parameters between hydrophilic and hydrophobic SAS may be similar (i.e. particle size, surface area and shape), RAC decided not to consider them in the CLH evaluation of the SAS-HMDS classification based on the following reasons:

i. significant differences exist both with regard to the chemical structure (free OH groups) and other physicochemical parameters such as surface chemistry, hydrophobicity, solubility (rate of dissolution/equilibrium solubility) and dispersibility

ii. the differences, mentioned above (and explained in more detail in the Supplemental information in the Background Document), can render hydrophilic SAS different in their biological and environmental reactivity/fate compared to hydrophobic SAS

iii. there is a lack of relevant data to support and justify possible read across between the hydrophilic and hydrophobic forms of SAS

In addition, it is noted that a similar grouping approach to that used by RAC has been widely accepted and used in the open literature both for human health and environmental hazards (see e.g. SCCS, 2019; Becker *et al.,* 2013; Pölloth, 2012; EPA, 2011; ECETOC, 2006; OECD SIDS, 2004).

It should be noted that although both the guidance on data requirements for nanomaterials and the updated guidance for grouping of nanoforms are still in preparation, there is enough evidence to justify the read across among the hydrophobic polymorphs of SAS included and discussed in the opinion. Moreover, the proposed read across is in accordance with the current version of the "Appendix R.6-1 for nanomaterials applicable to the Guidance on QSARs and Grouping of Chemicals, Version 1.0 May 2017".

Thus, **SAS-HMDS is the substance to which this CLH assessment applies and SAS-DDS and SAS-PDMS are sufficiently similar surface modified SAS, which are used as source substances in the read across assessment applied in this opinion**.

However, in order for the RAC to have a more rounded picture of the toxicological profile of SAS-HMDS, data on the hydrophilic SAS included in the CLH report referring to human health endpoints will be presented hereafter in each relevant hazard endpoint.

The source of the data supporting read across in the CLH report comes mainly from the CAR dossier and in one hazard endpoint (reproductive toxicity) the ECETOC (2006) and OECD SIDS (2004) reviews are mentioned. RAC has also noted the data from the ECETOC and the OECD SIDS reviews in their assessment for a number of endpoints, namely for acute toxicity, STOT SE and STOT RE. However, the various hydrophobic SAS polymorphs have also been extensively reviewed by Becker *et al.* (2013), Pölloth (2012), EPA (2011), ECETOC (2006), OECD SIDS (2004) and JRC (2013). In the additional key element section of the background document for each relevant

hazard endpoint, data from the aforementioned reviews of the open literature are also presented in order to have a more complete picture of the toxicological and ecotoxicological properties of the substance.

(Further details on the physicochemical characteristics of SAS and the in depth justification of the read-across are presented below).

Supplemental information - In depth analyses by RAC

There are three main types of silica (silicon dioxide), which are all included in the CAS number 7631-86-9: (1) crystalline silica, (2) amorphous silica (naturally occurring or as a by-product in the form of fused silica or silica fume), and (3) synthetic amorphous silica (SAS) (a schematic diagram of different polymorphs of silica is shown below). In the SAS category, depending on the method of production, there are four forms of silica under two different CAS numbers: (1) Wet process/CAS 112926-00-8 includes silica gel, precipitated silica and colloidal silica, (2) Thermal process/CAS 112945-52-5 includes pyrogenic (fumed) silica. All SAS can be chemically or physically surface treated (modified) to produce different surface treated silica, including the following: (1) Silica dimethicone silylate/CAS 67762-90-7, (2) silica dimethyl silylate/CAS 68611-44-9 and (3) Silanamine/CAS No 68909-20-6, which is the substance covered in the CLH report and this opinion.

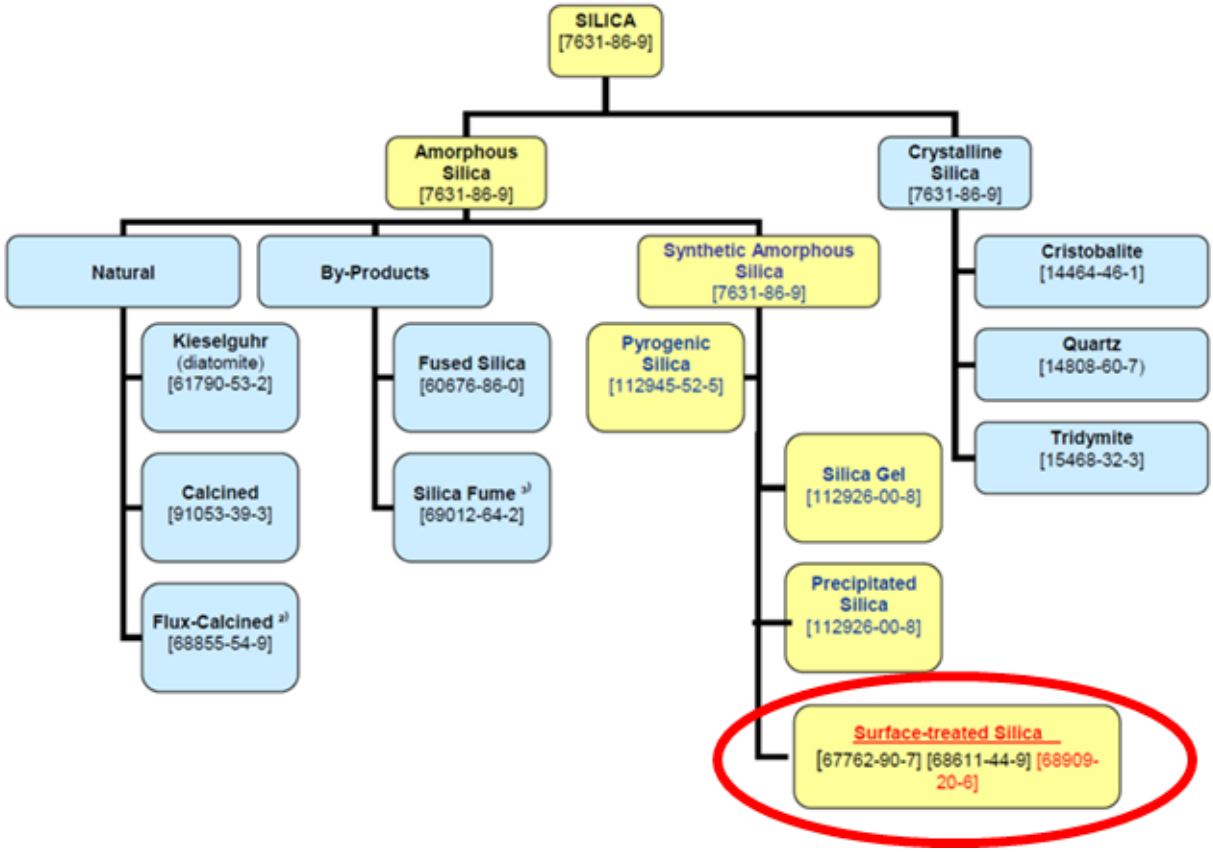
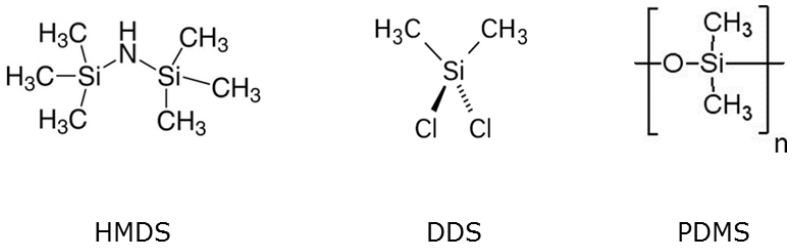


Figure. Schematic diagram of different polymorphs of silica

As described in the literature (ECETOC, 2006), a significant proportion of the global production of SAS is rendered hydrophobic to improve its affinity for and dispersion in non-polar materials (e.g. polymers, resins). In the following Figure the structures of three

common substances used to surface modify SAS are shown. The first compound forms mono-functional moieties upon hydrolysis, whereas the latter two give rise to bi-functional units, as shown below.



HMDS DDS PDMS

Figure. Structures of the three reagents used to surface modify SAS: Hexamethyldisilazane (HMDS; CAS No 999-97-3) → ≡Si–O–Si$(CH_3)_3$; Dimethyldichlorosilane (DDS; CAS No 75-78-5) → ≡Si–O–[Si$(CH_3)_2$–O–]$_{x\ =\ 1\ -\ 3}$; Polydimethylsiloxane (PDMS; CAS No 9016-00-6) → ≡Si–O–[Si$(CH_3)_2$–O–]$_{x\ =\ 3\ -\ 6(10)}$

The various forms of SAS are characterized by several physicochemical parameters such as SiO_2 content (%wt), carbon content (%wt), density (g/cm^3), loss on drying (%), water solubility (saturation) (mg/L, at ambient temperature and at 37°C and pH 7.1-7.4), pH (1:1 water:ethanol), specific surface area, B.E.T. (Brunauer, Emmett and Teller) (m^2/g), particle size measured by laser diffraction, behavior towards water etc, and the values thereof are reviewed in the literature (ECETOC, 2006; Pölloth, 2012; Becker *et al.* 2013; OECD SIDS, 2004).

These extra physicochemical parameters are necessary to describe a nanomaterial since size, shape and surface characteristics of a nanoform may cause the substance to exhibit a different behaviour compared to the non-nanoform of a material with the same composition (Guidance on information requirements and chemical safety assessment; Appendix R.6-1 for nanomaterials). It is not expected, though, that these parameters would account for toxicologically significant differences to necessitate inclusion in the SID additional identification parameters.

The physicochemical parameters of the various forms of SAS are shown in the following Table *(ECETOC 2006; Pölloth 2012; Becker et al. 2013; OECD SIDS 2004)*

Table: Compilation of physical and chemical properties of different SAS forms *(ECETOC 2006; Pölloth 2012; Becker et al. 2013; OECD SIDS 2004)*

Property (units)	**Pyrogenic**	**Precipitated**	**Colloidal**	**Gel**	**Surface Treated**
SiO_2 content (%wt)	≥ 99.8	> 95	≥ 99.5	-	≥ 99.8
Carbon content (%)	-	-	-	-	0.5-2
Loss on drying, (%)	< 2.5	5-7	» 2.5	2-6	< 2.5
Density (g/cm^3)	2.2	1.9-2.2	1.9-2.2	1.8-2.2	2.2-2.7
Water solubility (saturation), (mg/L) at 37°C and pH 7.1-7.4	144-151	141	Colloidal dispersion in water	127-141	115

pH (1:1 water:ethanol)	3.6-4.5	5-9	3.5-4.4 (4% w/v aqueous dispersion)	3-8	3.5-9
Specific surface area, B.E.T. (m^2/g)	50-500	30-800	50-380	250-1000	110-260
Behavior towards water	Hydrophilic	Hydrophilic	Hydrophilic	Hydrophilic	Hydrophobic
Particle size measured by laser diffraction					
Primary particle (nm)	5-50	5-100	1-10	1-10	5-20
Agreggate (µm)	0.1-1	0.1-1	0.1-1	1-20	0.1-1
Agglomerate (µm)	1-250	1-250	-	-	Mostly > 125

The specifications of the substance and the test materials to which this CLH assessment applies, as agreed by RAC, are presented in the following Table.

Table. Adjusted specifications of silanamine (substance to which this CLH assessment applies)	
Common name	Pyrogenic, Synthetic Amorphous Silicon dioxide, nano, surface treated silicon dioxide
Synonyms	Synthetic amorphous silica, Amorphous surface treated silicon dioxide
CAS-No.	68909-20-6
EINECS-No.	272-697-1
Other No. (CIPAC, ELINCS)	Not available
IUPAC Name	Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica; pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide
Chemical name	Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica; pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide
Other names	Silanamine, Silica silylate
Abbreviation used	SAS-HMDS
Molecular formula	$[SiO_2]n$-[OSi(CH3)3]m with n > m
	m corresponds to the surface treatment of silica with methyl groups. Thus, depending on the silica, n can be 10 times larger than m
Structure	Random arrangement of SiO_4 tetrahedron (base unit of the structure of the macromolecular network). The surface treated silica notified are synthetic amorphous silica with surface treatment Shape of primary particles: spherical

Molecular weight (g/mol)	Approximately 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 (carbon content actually only represents from 0.6 to 4% w/w).
Primary particle size (Transmission Electron Microscopy)	Specifications: 5-20 nm
Shape of primary particles (TEM)	Spherical
Specific surface area, B.E.T. (m^2/g)	90-290

In the following Tables, data specifications, publicly available by three of the main producers of hydrophobic silica, either evaluated or used as read across in the present opinion, are presented:

Table: *Data specifications, publicly available by Degussa, for the Degussa hydrophobic silica, either evaluated or used as read across in the present opinion*

Property (units)	**HYDRO PHOBIC SILICA**	**AEROSIL R812**	**AEROSIL R812S**	**AEROSIL R972**	**AEROSIL R974**	**AEROSIL R976**
CAS	68909-20-6 68611-44-9 67762-90-7	68909-20-6	68909-20-6	68611-44-9	68611-44-9	68611-44-9
Surface treatment	SAS-HMDS SAS-DDS SAS-PDMS	SAS-HMDS	SAS-HMDS	SAS-DDS	SAS-DDS	SAS-DDS
SiO_2 content (%wt)	≥ 99.8	≥ 99.8	≥ 99.8	≥ 99.8	≥ 99.8	≥ 99.8
Carbon content (%)	0.5-2	2.0-3.0%	3.0-4.0%	0.7-1.0	0.8-1.4	≈ 1.6
Loss on drying (%)	< 2.5	< 0.5%	< 0.5%	< 0.5%	< 0.5%	< 1.0%
Density (g/cm^3)	2.2-2.7	2.2-2.3	2.2-2.3	2.2-2.3	2.2-2.3	2.2-2.3
Water solubility (saturation), (mg/L) at 37°C and pH 7.1-7.4	115	-	-	-	-	-
pH 1:1 water ethanol	3.5-9	5.5-8.0	5.5-9.0	3.6-5.5	3.8-5.0	3.8-5.0
Specific surface area, B.E.T. (m^2/g)	110-260	230-290	195 - 245	90-130	150-190	225-275
Behavior towards water	Hydrophobic	Hydrophobic	Hydrophobic	Hydrophobic	Hydrophobic	Hydrophobic
Primary particle	5-20	6.9-8.6	6.9-8.6	12-16	12-16	12-16

(nm)						
Agreggate (µm)	0.1-1	0.1-1	0.1-1	0.1-1	0.1-1	0.1-1
Agglomerate (µm)	Mostly > 125	-	-	-	-	-

Table. Data specifications, publicly available by Cabot, for the Cabot hydrophobic silica either evaluated or used as read across in the present opinion

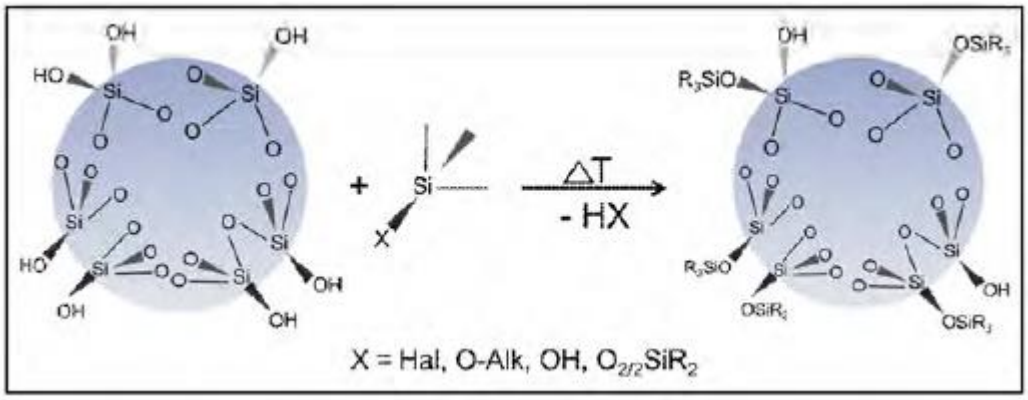
Property (units)	**CAB-O-SIL TS 530**	**CAB-O-SIL TS 5022**	**CAB-O-SIL TS 610**	**CAB-O-SIL TS 622**	**CAB-O-SIL TS 720**
CAS	68909-20-6	68909-20-6	68611-44-9	68611-44-9	67762-90-7
Surface treatment	SAS-HMDS	SAS-HMDS	SAS-DDS	SAS-DDS	SAS-PDMS
SiO_2 content (%wt)					
Carbon content (%)	4.25	2.5 ± 0.4	0.85		
Loss on drying (%)			< 0.5		
Density (g/cm^3)	2.2-2.3			2.2-2.3	2.2-2.3
Water solubility (saturation), (mg/L) at 37°C and pH 7.1-7.4					
pH (1:1 water:ethanol)	4.5-6.5		> 4.0	4.0-5.0	
Specific surface area, B.E.T. (m^2/g)	225	240 ± 30	125		120
Behavior towards water	Hydrophobic	Hydrophobic	Hydrophobic	Hydrophobic	Hydrophobic
Primary particle (nm)					
Agreggate (µm)	0.1-1.0	0.1-1.0	0.1-1.0	0.1-1.0	0.1-1.0
Agglomerate (µm)	-	-	-	-	-

***Table**: Data specifications, publicly available by Wacker, for the Wacker hydrophobic silica, either evaluated or used as read across in the present opinion*

Property (units)	**HDK® H30RM**	**HDK® H2000**	**HDK® H13L**	**HDK® H30**	**HDK® H18**
CAS	68909-20-6	68909-20-6	68611-44-9	68611-44-9	67762-90-8
Surface treatment	SAS-HMDS	SAS-HMDS	SAS-DDS	SAS-DDS	SAS-PDMS
SiO_2 content (%wt)	≥ 99.8	≥ 99.8	≥ 99.8	≥ 99.8	≥ 99.8
Carbon content (%)	3.0-4.6	2.3-3.2	0.6-2.2	1.4-2.6	4.0-5.2
Loss on drying, (%)	< 1.0	< 0.6	< 0.6	< 0.6	< 0.6

Density (g/cm^3)	2.2	2.2	2.2	2.2	2.2
Water solubility (saturation), (mg/L) at 37°C and pH 7.1-7.4					
pH (1:1 water:ethanol)	5.5-7.5	6.5-8.0		3.8-4.5	4.0-6.8
Specific surface area, B.E.T. (m^2/g)	200	200	110-140	270-330	170-230
Behavior towards water	Hydrophobic	Hydrophobic	Hydrophobic	Hydrophobic	Hydrophobic

Justification of the read-across between the different types of amorphous silica



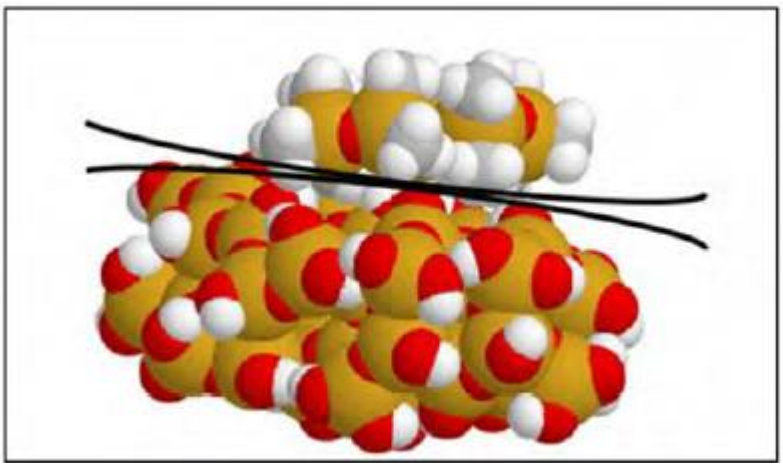


Figure. Schematic view of surface modification. Molecular model of surface treated SAS (shielding effect). Depicted in white are hydrogen atoms, in red oxygen atoms, in grey carbon atoms and in brown silicon atoms. The lower part of the figure represents a model of the silica surface, the upper part illustrates a model of the surface treatment agent shielding the surface area. Some Si-OH 20 groups are remaining unreacted.

The compilation for data specifications for the three hydrophobic silica either evaluated or used as read across in this report are shown in the following Table (values in the Table below are derived from the product specifications publicly available by all the commercial suppliers, as well as the

open literature).

***Table**: Compilation of data specifications for the three hydrophobic silica either evaluated or used as read across in the present opinion (ECETOC 2006; Pölloth 2012; Becker et al. 2013; OECD SIDS 2004)*

Property (units)	**SAS-HMDS**	**SAS-DDS**	**SAS-PDMS**
CAS	68909-20-6	68611-44-9	67762-90-7
Surface treatment	Hexamethyldisilazane HMDS	Dimethyldichlorosilane DDS	Polydimethylsiloxane PDMS
SiO_2 content (%wt)	≥ 99.8	≥ 99.8	≥ 99.8
Carbon content (%)	2.0-4.6 %	0.6-2.6	3.5-5.0
Loss on drying, (%)	< 1.0	< 1.0	< 1.0
Density (g/cm^3)	2.2	2.2	2.2
Water solubility (saturation), (mg/L) at 37°C and pH 7.1-7.4			
pH (1:1 water:ethanol)	4.5-8.0	3.6-5.0	4.0-7.0
Specific surface area, B.E.T. (m^2/g)	190-290	90-330	100-230
Behavior towards water	Hydrophobic	Hydrophobic	Hydrophobic
Particle size measured by laser diffraction			
Primary particle (nm)	5-20	5-20	5-20
Aggregate (µm)	0.1-1.0	0.1-1.0	0.1-1.0
Agglomerate (µm)	Mostly > 125	Mostly > 125	Mostly > 125
Read across outcome		*Used for read-across both for human health and environmental hazards*	*Used for read-across only for human health hazards*

There is evidence and results for certain parameters, such as chemical composition, particle size and shape, surface chemistry, surface area, solubility and rate of dissolution, hydrophobicity, zeta potential, dispersibility and dustiness in order to support the use of SAS-DDS and SAS-PDMS as source substances in a read across assessment for the classification of SAS-HMDS.

In order to evaluate human health toxicity, the biological reactivity and the toxicokinetics, availability in water systems included, are of major importance. Oral administration of SAS-PDMS to rhesus monkeys lead to expiration in the breath and excretion in the urine with a half-life of 24 hours, while after 92 hours more than 90% was recovered in the faeces. Inhalation of SAS-DDS by rats led to distribution in the lungs and mediastinal lymph nodes after 24 hours, while after three months > 80% of the test substance was eliminated (Becker *et al.*, 2013). The chemical structures of the various hydrophobic SAS forms (including the core and the surface) bear sufficient similarities among them in order to substantiate comparable biological reactivity (low hydroxylation state, di- and tri- methyl substituted silyl surface groups).

Toxicokinetic behaviour of a substance is dependent on water solubility. There is no established protocol to date to determine the solubility of hydrophobic powders and applying either the standard, or enhanced, OECD TG 105 methods either show a high degree of scatter in the results or no real result. However, a recent report of Roelofs and Vogelsberger (2004), reviewed in the Scientific Committee on Consumer Safety (SCCS) Opinion on the solubility of Synthetic Amorphous Silica (SAS), European Commission (2019), provided data on solubility and dissolution of

hydrophobic SAS, based on the working hypothesis that if surface treated SAS can be wetted, it should exhibit a certain solubility in water (the kinetics will be different from non surface treated SAS). This hypothesis is supported by the literature on the degradation behaviour of silica in water and biological systems (Croissant *et al.,* 2017; Cauda *et al.,* 2010). The modified NanoGenoTox protocol (NanoGenoTox, 2011) was used (but with 10% ethanol, instead of 0.5%). The results showed that all hydrophobic SAS products analysed so far exhibit a solubility between 100 and 160 mg/L in 10% ethanol/water. It is expected that other products not tested so far will be found to fit into that range. In that sense, taking into consideration the chemical structure similarity of the hydrophobic SAS (i.e. the dimethylsilyl moiety of SAS-DDS and SAS-PDMS should not result in differences in the aforementioned properties when compared to the trimethylsilyl moiety of SAS-HMDS) and the similar behaviour in water described above, the read-across for the human health hazards can be substantiated.

At the same time, caveats exist. More specifically, there is lack of data regarding the human toxicity endpoints in order to compare the biological stability, behaviour and reactivity of the three surface treated SAS used in the present opinion.

With regard to the environmental toxicity endpoints, under normal environmental conditions, silicon dioxide is an inert substance with no known degradation products. At ambient temperature and pH, hydrophobic SAS are practically insoluble in water. SAS are not volatile and have no lipophilic character. SAS are also photostable and there is no reason to believe that the slight differences in the surface of the SAS will alter the photoreactivity/stability of the SAS polymorphs. Therefore, the hydrophobic SAS will settle mainly into soils/sediments and weakly into water. SiO_2 is expected to combine with the soil layer or sediment in a way hard to distinguish due to the chemical similarity with inorganic soil matter. Thus, although there is no experimental data to show the same environmental behaviour/fate of the substance to which this CLH assessment applies with the read across substances, RAC believes that at standard environmental conditions the surface coatings should be more stable than in biological media, and their environmental reactivity should be similar, while any differences between the di- and the tri- substituted coatings should be insignificant.

Therefore, all three substances in the Table above are accepted for read across in this opinion, as they are similar structurally and physicochemically both with the evaluated substance and with each other. It has to be noted that the main limitation associated with SAS materials in general and specifically for the hydrophobic substances of interest for this CLH evaluation, is that depending on the method of preparation certain physicochemical properties may differ despite the fact that the same CAS number applies. According to the Guidance on information requirements and chemical safety assessment; Appendix R.6-1 for nanomaterials, such physicochemical properties (size, shape, surface area, solubility/hydrophobicity, zeta potential, dispersibility, dustiness) can affect exposure, toxicokinetics, fate and/or (eco)toxicological behaviour and thus the possible risk posed by nanoforms, they constitute the basic information to be considered for grouping and read-across.

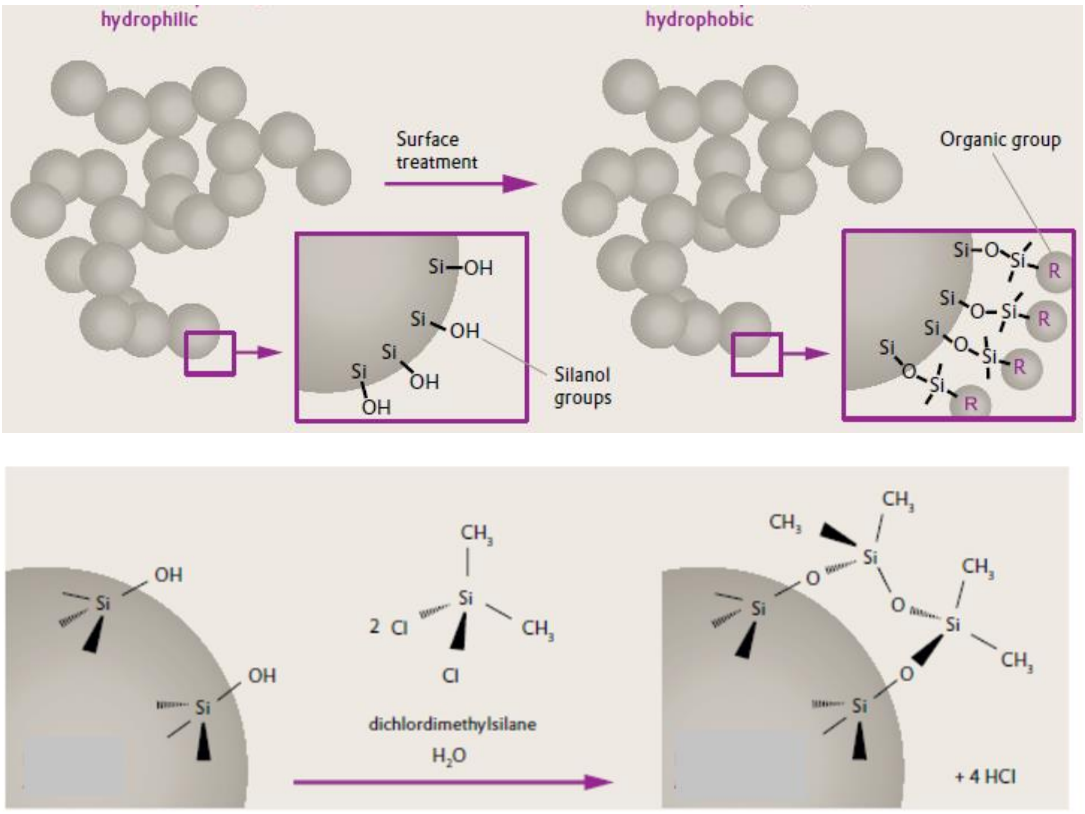


Figure. Structures of hydrophilic and hydrophobic SAS

Regarding the non-treated, hydrophilic SAS, although they are used as read across for certain hazard endpoints in the CLH report, RAC decides not to consider them in the CLH evaluation of the SAS-HMDS classification based on significant differences they present compared to the silanamine both on the chemical structure (free OH groups, Figure above) and on certain physicochemical parameters. More specifically:

- Surface chemistry: The term surface chemistry indicates the chemical composition at the surface of the particles as a result of chemical coating and/or surface treatment of the particle. Surface chemistry influences dissolution behaviour and agglomeration behaviour of nanoforms. Considering hazard endpoints, the surface chemistry of a nanoform affects its reactivity and systemic absorption. Surface modification may determine which biomolecules adhere to the nanoform, its distribution and cellular uptake, and its toxic effects. In the environment, surface chemistry will influence sorption to environmental or biological media and the reactivity of a nanoform. Thus, RAC believes that the surface chemistry of the hydrophilic and the hydrophobic forms of SAS differ substantially, as in the former case the surface consists of Si-OH (silanol) groups and in the latter of $-SiO(Me)_2$ and $-Si(Me)_3$ units. Moreover, there is no data to compare and prove that the surface chemistry of the hydrophilic and the hydrophobic polymorphs of SAS is similar.
- Hydrophobicity: Surface treatments converting hydrophilic into hydrophobic silica can only be expected to decrease the solubility of the materials. Hydrophobicity can influence agglomeration and sorption, as well as 'dispersibility in biological media' and dustiness. In the two SAS polymorphs the hydrophobicity is very different since the Si-OH, $-SiO(Me)_2$ and $-Si(Me)_3$ surface groups affect the behaviour of the two SAS forms. Moreover, this is the

purpose of the surface modification of SAS, to alter the surface behaviour of SAS from hydrophilic to hydrophobic. In Figure below the behaviour of hydrophilic and hydrophobic SAS in water is shown.

Figure: Water with hydrophilic silica (left) and hydrophobic silica (right)

- Solubility: Rate of dissolution / Equilibrium solubility:

  The rate of dissolution depends on factors including, but not limited to the chemical composition, particle size, coating, surface treatment, stability, manufacturing process, and biological environment. The rate of dissolution gives information on how many ions/molecules are released from the particle over time. The ions/ molecules released may also dictate the toxicity of the nanoforms, which will be an important aspect of the CLH evaluation. In EPA (2011) hydrophobic SAS are reported as practically insoluble in water at room temperature, which is not the case with hydrophilic SAS. The surface treated, hydrophobic silica in general had a lower solubility compared to the hydrophilic SAS, due to its hydrophobic surface and consequent reduced wetting of its surface in aqueous systems. Although in the SCCS (2019) report it is stated that temperature plays an important role in the solubility behaviour of hydrophobic and hydrophilic SAS and that at 37°C in a medium mimicking plasma, hydrophobic and hydrophilic SAS present comparable solubility, the hydrophobic SAS have almost 40% lower solubility. At standard environmental conditions, though, the solubility of hydrophobic SAS is negligible ($< 10^{-4}$ mg/L; EPA, 2011). Therefore, RAC believes that hydrophilic SAS will always have higher solubility than hydrophobic SAS (the higher the temperature the lower the difference in solubility) and the dissolution rate will be different between hydrophilic and hydrophobic forms of SAS. As a consequence, hydrophilic SAS are not suitable for read across at least for the environmental endpoints, while for human health endpoints it could possibly lead to over-classification.

- Dispersibility:

  This parameter can influence the degree of environmental transport and (environmental) exposure. Furthermore, this parameter may influence the degree of internal exposure (particularly by the oral route; however particle dispersibility also affects nanomaterial mobility within the lung and hence its potential for systemic uptake). Dispersibility, is one of the fundamental differences between hydrophilic and hydrophobic SAS, especially in aqueous media.

Therefore, RAC has decided to use **only the hydrophobic polymorph for classification purposes**.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Large-scale production and use of amorphous silica nanoparticles (SiNPs) have increased the risk of human exposure to SiNPs, while their health effects remain unclear. STOT RE is proposed by industrial minerals producers (see their website at https://www.crystallinesilica.eu/content/classification-and-labelling-rcs). Action is proposed in view of the divergences in notifications from the C&L inventory where 99% of the notifiers (n=1841) do not classify.

5 IDENTIFIED USES

Under regulation (EU) 528/2012, "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):

http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R0795&from=EN

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

6 DATA SOURCES

The information from the Competent Authority Report of the pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide are included in the dossier.

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	Expert assessment	Statement

Property	Value	Reference	Comment (e.g. measured or estimated)
	water. Due to the fonctionalisation of the surface it is expected that functionalised Aerosil is not susceptible to hydrolyse in monosilicic acid.		
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	
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 below the table		
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	Not applicable	-	
Viscosity	Not applicable	-	

Particle size distribution:

Silica is produced as very small particles called primary particles that have potential to aggregate. Aggregate are particle 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.

Different studies were submitted on different shear forces to characterize the active substances. The curves are submitted but raw data are not submitted on each volume fraction implying blanks in tables below:

Table 8: particle size distribution of silicon dioxide surface treated under different condition

Volume fraction %	d_{10}	d_{50}	d_{90}	Proportion <10µm
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%

It will be consider 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 (d50 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 is formulated in Indispron D110, the measured size of particles increases. Additional microscopy data are submitted to confirm the particle size distribution in Indispron D110. No data is 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 aerosol R812S using BET method.

The range on 5 batches was found in the range of 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

8.1 Explosives

The substance is an inorganic, inert solid with mineral character, almost fully oxidised, therefore, no structural alerts for explosivity (silica derivative).

8.2 Flammable gases (including chemically unstable gases)

Not relevant

8.3 Oxidising gases

Not relevant

8.4 Gases under pressure

Not relevant

8.5 Flammable liquids

Not relevant

8.6 Flammable solids

The substance is an inorganic, inert solid with mineral character, almost fully oxidised, therefore, no structural alerts for flammability (silica derivative).

8.7 Self-reactive substances

The substance is an inorganic, inert solid with mineral character, almost fully oxidised, therefore, no structural alerts for self-reactive behaviour (silica derivative).

8.8 Pyrophoric liquids

Not relevant

8.9 Pyrophoric solids

The substance is an inorganic, inert solid with mineral character, almost fully oxidised, therefore, no structural alerts for pyrophoric properties (silica derivative).

8.10 Self-heating substances

The substance is an inorganic, inert solid with mineral character, with high melting point.

No self-heating behaviour expected.

8.11 Substances which in contact with water emit flammable gases

The substance is an inorganic, inert solid with mineral character, almost fully oxidised, therefore, no flammable gases is expected to be emitted with contact with water.

8.12 Oxidising liquids

Not relevant

8.13 Oxidising solids

The substance is an inorganic, inert solid with mineral character, almost fully oxidised, therefore, no structural alerts for oxidising properties (silica derivative).

8.14 Organic peroxides

Not relevant

8.15 Corrosive to metals

Not relevant

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) did not propose classification of silanamine based on the fact that the substance is an inorganic, inert solid with mineral character (silica derivative), is almost fully oxidised, with a high melting point and therefore has no structural alerts for explosive, flammable, self-reactive, pyrophoric, self-heating or oxidising properties. Moreover, no flammable gases are expected to be emitted in contact with water.

Comments received during public consultation

No comments were received about the physical hazards of the substance during public consultations.

Assessment and comparison with the classification criteria

RAC supports and agrees with the DS's proposal for **no classification of silanamine regarding physical hazards**.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

There is no toxicokinetics data specifically related to the substance covered by this dossier. The lack of systemic effects reported in the toxicity studies can be due to a lack of systemic absorption.

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

No information is available regarding the toxicokinetic profile of the substance.
No systemic effect has been observed.

10 EVALUATION OF HEALTH HAZARDS

Read-across between the different types of amorphous silica

The substance under consideration in the Biocidal Product Dossier and relevant for the claimed application consists of reaction product of synthetic amorphous silica treated with hexamethylsilazane (leading to a silica characterised by CAS No 68909-20-6 and marketed as Aerosil R 812 and Aerosil R 812S). This does not apply to other silica, as amorphous non surface-treated silica or crystalline silica. In this dossier, an X-ray analysis showed that Aerosil R 812 and R 812S have a content of crystalline silica < 0.1%.

Several toxicological studies (acute inhalation study, repeated dose toxicity studies by oral route and inhalation, carcinogenicity study by oral route and one-generation study by oral route) were performed with Aerosil R 972 or Aerosil R 974 which are reaction products of dichlorodimethyl-silane with silica characterised by CAS No 68611-44-9.

For repeated oral toxicity endpoints, the studies were performed with Aerosil R 972. The difference between Aerosil R 972 and Aerosil R 812/812S is the nature of the surface-treatment (hexamethylsilazane for CAS 68909-20-6 (HMDZ) and dichlorodimethylsilane for CAS 68611-44-9). The chemical groups added by the reaction have no particular activity by themselves impacting the repeated toxicity of both Aerosil.

Based on Substane Evaluation Reports avialbel for HMDZ and dichlorodimethylsilane (2015), concerns were specifically related to environnement. Classification described in the reports suggest that both substances shere similar toxicological properties (irritant properties) without any sufficient hazards triggering a classification STOT RE.

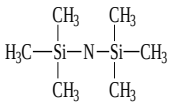
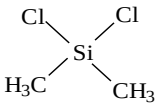
Similar physico-chemical properties such as non solubility in water and stability to hydrolysis are reported between these two types of silica. Therefore, no impact on systemic toxicity after oral exposure is expected.

Table 10.1. Identity of the tested materials

Common name, Synonyms	**Pyrogenic, Synthetic Amorphous Silicon dioxide, nano, surface treated silicon dioxide** Synthetic amorphous silica, Amorphous surface treated silicon dioxide	
CAS-No.	68909-20-6	68611-44-9
EINECS-No.	272-697-1	271-893-4
Other No. (CIPAC, ELINCS)	/	/
IUPAC Name	Reaction products of 1,1,1-trimethyl-N-(trimethylsilyl)-silanamine with silica	Reaction products of dichlorodimethyl-silane with silica
Chemical name	Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica	Silane, dichlorodimethyl-, reaction products with silica
Trade names used	Aerosil R 812 S * Aerosil R 812	Aerosil R 972 Aerosil R 974 Aerosil R 976
Molecular formula	$[SiO_2]_n$-$[OSi(CH_3)_3]_m$ with n>m	$[SiO_2]_n$-$[OSi(CH_3)_2]_m$ WITH N>M
	m corresponds to the surface treatment of silica with methyl groups. thus, depending on aerosils notified n can be from more than 10 times higher than m.	
Structure	Random arrangement of SiO_4 tetrahedron (base unit of the structure of the macromolecular network). The surface treated silica notified are synthetic amorphous silica with surface treatment Shape of primary particles: spherical	
Molecular weight (g/mol)	Approx 60.08 g/mol (which is the molecular weight of one unit of SiO_2) The surface modification does not significantly affect a lot the molecular weight of the substance which is slightly higher (carbon content actually only represents from 0.6 to 4% w/w).	
Primary particle size (TEM)	Experimental data : 6.9-8.6 nm Specifications: 6.9-8.6 nm	No experimental data Specifications: 12-16 nm
Shape of primary particles (TEM)	spherical	spherical

Table 10.2. Production process: (Degussa 2003a: Technical Bulletin Fine Particles)

Synthetic amorphous *hydrophobic* silica are produced by surface treatment of the synthetic amorphous *hydrophilic* silica:

Step of production process	Aerosil R 812 and Aerosil R 812 S [CAS No. 68909-20-6]	Aerosil R 972, R 974, R 976 [CAS No. 68611-44-9]
1. Silica production, pyrogenic, hydrophilic [CAS 112945-52-5]	The raw material $SiCl_4$ (tetrachlorosilane) or as a mixture with other chlorosilanes of alkylchlorosilane is mixed at gaseous stage with air and hydrogen and is burned in a flame at about 1000 °C ("flame hydrolysis"), according to the following equation (pyrogenic process): $SiCl_4 + 2\ H_2 + O_2 \rightarrow SiO_2$ (polymer, hydrophilic, amorphous) + 4 HCl	
2. Chemical after-treatment of the hydrophilic amorphous silica: The surface-attached free hydroxyl groups (silanol groups) are irreversibly replaced by organic residues such as methyl groups	Surface modification with hexamethylsilazane [CAS No. 999-97-3]  This results in a trimethylsilyl-surface modified silica which is highly hydrophobic. The main difference between Aerosil R 812 and R 812 S is the density of superficial methyl groups which is slightly higher in Aerosil R 812 S (2-3% for R812 and 3.0-4.0 for R812S). The specific surface area (260 m²/g for R812 and 220 m²/g for R812S) is also a data which differentiates the Aerosils R 812 and R 812 S, but those two values remain in the same range.	Surface modification with dichlorodimethylsilane [CAS No. 75-78-5]  This results in a dimethylsilyl-surface modified silica, which are somewhat less hydrophobic than Aerosil R 812 S due to the lower density of superficial methyl groups. The difference between Aerosil R 972, Aerosil R 974 and Aerosil R 976 is the density of superficial methyl groups which is slightly lower than the Aerosils R 812 and R 812 S and the specific surface area

For the specific concern of inhalation, the studies were performed with Aerosil R 974. In this case, the main relevant physico-chemical parameter influencing absorption is the particle size. Under conditions of normal handling and use, primary particle of surface-treated silica is not expected and the aggregates are considered as the smallest stable particles. In this context, the particle size distributions of aggregates of Aerosil R 812/R 812S and Aerosil R 974 were compared in high shear forces conditions. The values are in the same order of magnitude (peak and shapes of curves). This suggests that results from inhalation tests on Aerosil R 974 can be extrapolated to Aerosil R 812/R 812S.

Table 10.3: Comparative table between the different AEROSIL presented in the dossier

	Synthetic amorphous silica, surface treated				
	CAS No. 68909-20-6		**CAS No. 68611-44-9**		
	R 812 S	**R 812**	**R 972**	**R 974**	**R 976**
Specific surface area (BET) [m^2/g]	220 ±25	260 ±30	110 ±20	170 ±50	250 ±25
Average primary particle size					
Specification	7 nm	7 nm	16 nm	12 nm	No data
experimental data	7 -8 nm	5 nm	No data	No data	No data
Particle size **high shear force → aggregates**					
range (d5-d95)	88-240 nm	No data	120-240 nm	50-300 nm	No data
median (d50)	150 nm	No data	179 nm	120 nm	No data
Particle size **low shear force in ethanol → agglomerates**					
range (d5-d95)	2-32 µm	No data	2-23 µm	No data	No data
median (d50)	11.2 µm	No data	4.4 µm	No data	No data
Particle size **powder**					
range (d5-d95)	6-90 µm	7-130 µm	No data	No data	No data
median (d50)	23µm	30 µm	No data	No data	No data

Teratogenicity studies performed with a synthetic amorphous non surface-treated silica gel were also submitted. The aim of the surface modification is to block the silanol group in order to reduce the affinity of silica for water. Therefore, it is expected that surface-treated silica would not be better absorbed by oral route than non surface-treated silica. Furthermore, the lack of systemic effects of both surface treated silica and non surface-treated silica in oral toxicity studies (based on data submitted in this dossier for the first and on literature for the latter) supports a read-across for systemic toxicity. However, for local pulmonary endpoints, it was agreed at Biocidal Technical Meeting II 2011 that no read-across between surface-treated and non surface-treated silica should be considered since it could not be concluded that these types of silica are technically equivalent.

It has to be noted the substance is a nanoparticle, however the available studies are not designed to assess specifically the toxicity linked to this property.

10.1 Acute toxicity - oral route

Table 10.1.1: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
OECD 401 (1981)	Rat, Wistar, 5 m, 5 f	Aerosil R 812	2 000 mg/kg bw, single dose	> 2 000 mg/kg bw	IIIA6.1.1

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Acute oral exposure to Aerosil R 812 is void of acute toxic adverse effects (LD_{50} > 2000 mg/kg bw). No signs of intoxication were noticed after 14 days of observation.

10.1.2 Comparison with the CLP criteria

The LD_{50} in rats is higher than 2000 mg/kg bw.
This value is out of the range for classification under regulation (EC) 1272/2008.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the results of the acute oral toxicity study, no classification is proposed for the active substance.

10.2 Acute toxicity - dermal route

No study was provided for acute toxicity by dermal route.

Information from the skin irritation study performed with Aerosil R 812 (see section below) could suggest a low dermal toxicity of Pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide since no mortality was observed at the dose of 0.5 g per animal.

10.3 Acute toxicity - inhalation route

Table 10.3.1: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, , form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value LC_{50}**	**Reference**
No data on the method	Rat, Wistar, 5 m, 5 f	Aerosil R974 56% of the particles had	477 mg/m^3 [analytical,] 4 h	> 477 mg/m^3	IIIA6.1.3

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, , form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value** LC_{50}	**Reference**
		an aerodynamic diameter <5µm (respirable)			

Table 10.3.2: Summary table of other studies relevant for acute inhalation toxicity

Type of study/data	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Mechanistic study on local effects, inflammation reaction model, no GLP	Aerosil R 812 S	Rat, Wistar, 10 f/dose Intra-tracheal application 0.15, 0.30, 0.60, and 1.2 mg dust/lung single dose with an observation period of 3, 21, and 90d	All doses: Acute-phase reaction (3 d): reversible increase in inflammation markers from bronchoalveolar lavage	IIIA6.10

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

Inhalation of respirable Aerosil R 974 provokes an inflammatory tissue reaction.
Similar inflammatory reactions have been described for other dusts (LC_{50} > 477 mg/m^3 [maximum attainable concentration]).

After a single *intratracheal* application of Aerosil R 812S, inflammation was assessed in the bronchoalveolar lavage, by counts of neutrophils, macrophages, total cells, and the expression of specific proliferation proteins and TNF-alpha.
At day 3, a significant transient increase of inflammatory markers was observed from the lowest tested dose (0.15 mg dust/lung) with a severity clearly dose-dependent.
At the dose of 0.6 mg dust/lung, the intensity was somewhat lower, but comparable to that of quartz at the same dose. Return to normal levels was reached within 21 days for all doses of Aerosil R 812S (highest dose 1.2 mg/lung), in contrast to quartz that induced a progressively chronic inflammation, not reversible within 90 days of observation. Furthermore, after a single exposure to Aerosil R 812S, no sign of fibrosis was evident in the lungs at 90 days post-exposure.

10.3.2 Comparison with the CLP criteria

The Lc_{50} in rats is higher than 477 mg/m^3 (corresponding to 0.48 mg/L).

The design of the study did not allow to determine a LC_{50}. Therefore, it is not possible to conclude whether the substance is acutely toxic by inhalation under regulation (EC) 1272/2008.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on the results of the acute inhalation toxicity study, no classification is proposed for the active substance.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity

The DS proposed no classification for the acute oral toxicity of SAS-HMDS (Aerosil R812) based on a negative OECD TG 401, GLP compliant study with Wistar rats (A6.1.1). The LD_{50} was estimated to be higher than 2000 mg/kg bw.

Acute dermal toxicity

No study was provided for acute toxicity by dermal route. However, the DS proposed no classification for acute dermal toxicity because data from the skin irritation study performed with SAS-HMDS (Aerosil R812) suggested a low dermal toxicity of pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide, since no mortality was observed at the dose of 0.5 g per animal (about 200 mg/kg bw).

Acute inhalation toxicity

Two studies were relevant to assessing the acute inhalation toxicity of silanamine. One was a non-guideline, GLP compliant study, with reliability 2 (Klimisch), with the read across substance SAS-DDS (Aerosil R974) and a non-guideline, non GLP, reliability 2 mechanistic study following a single intratracheal injection of SAS-HMDS (Aerosil R812S). No mortalities were observed at the maximum concentration attained which was 477 mg/m^3 (0.48 mg/L) in the first study. Due to the design of the study the dose used is well below the suggested concentration for an aerosol (5 mg/L) according to OECD TG 403. The mechanistic study showed an increase of the inflammatory markers which were fully reversible within 21 days. The DS proposed no classification due to lack of data, since no LC_{50} was determined.

Comments received during public consultation

No comments were received during public consultation conducted from 04.03.2019 to 03.05.2019.

During its December (2019) meeting, the Committee for Risk Assessment (RAC) concluded that silanamine should be classified as Acute Tox 2 via the inhalation route (H330) with an ATE of 0.45 mg/L, as well as STOT RE 2; H373 (lungs, inhalation). Since some of the studies leading to the acute toxicity classification were not summarised in the CLH report, an ad hoc consultation of the documents in which these studies have been summarised was launched from 03.02.2020 to 17.02.2020 and the comments received on acute toxicity endpoint are summarised below.

There were 13 comments received, 8 from industry and 5 from individuals. The comments

focused on two different aspects of the classification process. First, industry commented on procedural issues relating to the specific substance and secondly challenged the scientific interpretation of the data regarding the acute inhalation endpoint.

Scientific Issues

- Industry indicated that they had initiated a new mechanistic study on acute inhalation of SAS within the framework of the REACH substance evaluation.
- The majority of the studies were conducted before the release of OECD Guideline 403 (September, 2009). As a result, the methodology used does not follow current standards for assessing acute inhalation toxicity in general.
- Industry also challenged the reliability scores of the studies used, as reviewing independent experts recently downgraded substantially the reliability of these studies.
- The particle size distribution of the SAS used in the inhalation toxicity testing is significantly reduced to fulfil testing guideline requirements (MMAD < 4 µm) to generate respirable particles and therefore is widely different from the particle sizes (MMAD > 100 µm) of commercially used SAS. Thus, industry concludes that the test substance has no relevance for exposure to humans.
- Due to the tendency of SAS to agglomerate, the small respirable particles that reach the alveoli, re-agglomerate and form larger particles which cause suffocation of the animals. Thus, industry considers that the lethality is due to suffocation and does not represent an intrinsic property of the silanamine and moreover does not represent real life conditions. The mechanistic study on acute inhalation of SAS initiated within the framework of the substance evaluation could add evidence to the suggested suffocation mechanism.

Additional key elements

Acute oral toxicity

In the ECETOC (2006) review, studies for acute oral toxicity using all three hydrophobic surface treated SAS (SAS-HMDS, SAS-DDS, SAS-PDMS, each from a different supplier) are presented and these did not induce acute oral toxicity up to the highest dose tested. The LD_{50} varied from > 2000 mg/kg bw up to > 5000 mg/kg bw.

Acute dermal toxicity

In the Becker *et al.* (2013) review, SAS-DDS (2000 mg/kg bw in propylene glycol) applied in a single dose to the skin of Wistar rats (n = 5/sex) for 24 h caused no mortality. No clinical signs were observed. Necropsies resulted in no significant findings.

Acute inhalation toxicity

There were several additional acute inhalation studies in the open literature with all three forms of hydrophobic SAS. A selection of the studies are summarised in following Table. The majority of the studies were performed before the OECD Guideline 403 was adopted (September, 2009). The studies mentioned have been evaluated by ECETOC and reliability codes have been assigned to them (ECETOC, 2006). ECETOC's code of reliability is based on the Klimisch scale. The ECETOC review was published in 2006 and the reliability assessment of all studies included in the review

was not disputed by any interested parties. Moreover, the specific studies are internationally recognized having been referenced in many reviews. RAC is not aware of the reliability evaluations having previously been questioned. Neither revised evaluation criteria nor a list of the deficiencies recognized in these studies have been provided. However, for the studies in the ECETOC review, only the results of the studies and not the actual raw data are available to RAC and in some cases the details of the experimental design and performance are vague or unknown, RAC has decided not to use the reliability evaluation of the studies performed by ECETOC, and use all studies from the open literature in the weight of evidence approach. Studies summarised in the CLH report and in the CAR are given a Klimisch reliability score and their raw data are available. Therefore, RAC decided to use this rating only.

Table: *Acute inhalation toxicity studies with all three forms of hydrophobic SAS available in the open literature*

A/A	**Species / Reference/ Year of the study***	**Method, Test substance** **(TG and GLP information are from ECETOC, 2006)**	**LC_{50} (mg/L)**	**Other observations**
1	BR Rat / ECETOC, 2006, Becker *et al*., 2013 / Cabot, 1982	*Guideline study with acceptable restrictions* 5/sex Single dose: 2280 mg/m^3 Exposure: 1h Particle size/MMAD**: 0.15 µm SAS-DDS (Cabot) Control group	> 2.28	No mortalities observed No LC_{50} determined Clinical signs (during and after exposure): Irregular breathing[1] After treatment: poor coat quality and alopecia in females
2	Wistar rats / ECETOC, 2006, Becker *et al*. 2013, EPA, 2011 / Cabot 1994	*Comparable to guideline study* 5/sex Doses: 210, 540, 2100 mg/m^3 Exposure: 4h Particle size/MMAD: 0.8-1 µm/ 1.175-1.275 µm Surface Area: 130 m^2/g SAS-DDS (Cab-O-Sil TS610)	0.45	Mortality Dose (mg/m^3): 210 / 540 / 2100 Mortality: 0/10 / 7^/10 / 10#/10 ^ *animals died during exposure* # *all animals died within 2.5 hours* Other findings **210 mg/m^3** During exposure: closed eyes[2], laboured breathing[1], licking inside of mouth and laying on back After exposure: sporadic instances of few faeces, anorexia, chromodacryorrhoea[2], laboured breathing[1], wetness of the nose/mouth area[3], diarrhoea and transient decreases in body weight gain Necropsy findings: darker lungs than normal[4],

				white and red areas in lungs[4]. **540 mg/m³** During exposure: closed eyes[2], red staining of the nose/mouth area[3], fur coated with test substance, laboured breathing[1], respiratory distress and hunched position[1] After exposure: lethargy[2], piloerection, dyspnea[1], ptosis[2], few faeces, eyes crusting/lachrymation[3], unkempt appearance, wetness of the anogenital area and eye opacity. From days 4 to 14, all surviving rats appeared normal. Body weights in the surviving females decreased on day 7 but had recovered by day 14. Necropsy findings (dead animals): Wetness of the anogenital area, opaque eyes[2], lungs larger and darker than normal with red areas[4], white material in the nasal turbinates[3] and red areas in the intestines. Necropsy findings (survivors): Lungs darker than normal with red and white areas[4]. **2100 mg/m³** Pre-death signs: few faeces, closed eyes[2], wetness and red staining of the nose/mouth area[3], laboured breathing[1], respiratory distress and hunched position[1] Necropsy findings: eye opacity[2], lungs larger than normal with red areas[4], and white material in the nasal turbinates[3]
3	Wistar rats / ECETOC, 2006 / Cabot 1994	*Comparable to guideline study* 5/sex Dose: 90, 840 mg/m³ Exposure: 4h Particle size/MMAD: 0.95-2.15 µm Surface Area: 300 m²/g SAS-HMDS (Cab-O-Sil TS530)	0.09-0.84	Mortality Dose (mg/m³) | 90 | 840 Mortality | 0/10 | 10/10 Other findings The effects seen during and after exposure were similar to the study above.
4	BR rats / Becker, 2013, EPA 2011 /	5/sex, high dose 7/sex Dose: 520, 1120,	0.52-1.12	Mortality Dose (mg/m³) | 520 | 1120 | 2790

<table>
<tr>
<td></td>
<td>Cabot 2003 (revised)</td>
<td>2790 mg/m^3
Exposure: 4h
Particle size/MMAD: 1.24 µm
SAS-DDS</td>
<td></td>
<td>
<table>
<tr><td>Mortality</td><td>0/10</td><td>10/10</td><td>14/14</td></tr>
</table>
All animals that died, died during exposure
Other findings
520 mg/m^3
Normal body weight gain.
During exposure: decreased, irregular breathing[1]
After exposure: increased breathing rates[1], laboured breathing[1] and blepharospasm[2], all of which resolved in four days.
Necropsy findings: Lungs filled with foam[4]
1120 mg/m^3
During exposure: decreased, irregular breathing[1]
Necropsy findings: Hemorrhage[4], reduced elasticity in the lungs[4], soiled fur, white powder in the nasal cavity
2790 mg/m^3
During exposure: decreased irregular breathing[1]
Necropsy findings: Petechiae in the lungs, obstructive lumps of white particles and slime in the nose, and haemorrhage in the nasopharynx.
Histopathology[4]: Erythrocytes and oedema in alveoli, epithelial lining interrupted or flattened and scarce goblets cells. The lumina of the nasopharynx, larynx and bronchi/bronchioles contained large quantities of pale eosinophilic material mixed with nucleated cells and erythrocytes. The material filled the entire lumen in the smaller bronchioles.
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<tr>
<td>5</td>
<td>SD rats / ECETOC, 2006 / Wacker, 1996</td>
<td>Good laboratory practice guideline study (OECD, EC, EPA, FDA, etc.)
5/sex
Dose: 350, 770, 2530, 5300 mg/m^3
Exposure: 4h
Particle size/MMAD: < 0.2 µm
Surface area: 130 m^2/g
SAS-HMDS
HDK SKS130</td>
<td>1.65</td>
<td>
Mortality
<table>
<tr><td>Dose (mg/m^3)</td><td>350</td><td>770</td><td>2530</td><td>5300</td></tr>
<tr><td>Mortality</td><td>0/10</td><td>0/10</td><td>10[#]/10</td><td>10[#]/10</td></tr>
</table>
[#] all animals died within 4 hours
Other findings
At necropsy, severe red discoloration of the lungs[4] was observed in all animals that had died during exposure.
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<td>6</td>
<td>SD rats / ECETOC, 2006 /</td>
<td>Good laboratory practice guideline study (OECD, EC, EPA, FDA, etc.)</td>
<td>> 2.2</td>
<td>
Mortality
<table>
<tr><td>Dose</td><td>900</td><td>2200</td></tr>
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	Wacker, 1996	5/sex Dose: 900, 2200 mg/m^3 Exposure: 4h nose only Particle size/MMAD: 7.2-7.7 µm Surface area: 130 m^2/g SAS-HMDS HDK SKS130		(mg/m^3) | | | Mortality | 0/10 | 4/10 <u>Note</u> Same test as above with the same SAS with higher MMAD was carried out. <u>Other findings</u> **900 mg/m^3** 1/5 male and 2/5 females showed trace red discoloration of the lungs[4] **2200 mg/m^3** At necropsy, the animals that had died exhibited severe discoloration of the lungs[4]; all other animals were within normal limits

* *All open literature references, where the study is reviewed are mentioned, along with the Industry performing the study and the year of the study*

** *Becker et al. (2013) provides particle size dimensions in µm; ECETOC (2006) provides particle size MMAD (Mass Median Aerodynamic Diameter calculated by Cascade impactor) in µm; MMAD is defined as the aerodynamic diameter at which 50% of the particles by mass are larger and 50% are smaller*

[1] *Clinical signs of various pathologies possibly associated with respiratory tract abnormalities*

[2] *Clinical signs associated with peripheral/ autonomous nervous system*

[3] *Clinical signs associated with upper respiratory tract irritation*

[4] *Findings associated with respiratory tract abnormalities*

In the ECETOC review, two other studies on rats (4h exposure) are discussed using SAS-DDS by the producing company Wacker (HDK SKS 300) with the same surface area (300 m^2/g), but different particle size/ MMAD (< 0.1 µm and 7.0-7.1 µm, respectively). A three-dose scheme was applied in the first study (90, 350, 5000 mg/m^3) and a two-dose scheme (400, 600 mg/m^3) in the second, respectively. No original data on deaths were provided. The authors stated only the calculated LC_{50} values (0.09 and 0.5 mg/L, respectively), and concluded that the results indicated that the number of particles per specific surface area is responsible for the observed effects. Similar findings were obtained on studies #5 and #6 of the Table above, but the results are less pronounced (LC_{50} = 1.65 vs LC_{50} > 2.2 mg/L, respectively).

Reference to a mechanism of suffocation via obstruction of the airways due to agglomerate formation has been recently expressed by Industry in order to account for lethality observed in the acute toxicity studies by inhalation. No pathological findings, though, were reported supporting this mechanism. For example, tardieu spots on the lungs should have been reported in the pathology investigations. Moreover, there were no clinical signs associated with suffocation reported in any of the studies available for acute inhalation toxicity. In addition, the same clinical findings of difficulties in breathing are observed both in single dose experiments at non-lethal doses and in repeated dose toxicity studies, where the lungs are consistently the target tissue. The majority of clinical observations were reversible. Suffocation would not be a reversible effect with reversible clinical manifestations. The cluster of the histopathological findings point rather to acute respiratory distress syndrome due to high inflammation attack than to suffocation, as is discussed in the STOT SE and STOT RE sections of this opinion.

Assessment and comparison with the classification criteria

Acute Oral Toxicity

Table: Acute oral toxicity studies in the CLH report

Species / Reference/ Year	Method, Test substance	LD_{50} (mg/kg bw)	Other observations
Wistar Rat / A6.1.1 / Degussa (Industry) 1981	OECD TG 401, GLP 5/sex/concentration One dose: 2000 mg/kg bw SAS-HMDS (Aerosil R812)	> 2000	No mortalities were observed. Clinical signs: During the first 5 hours following dosing, symptoms included slight sedation, slight dyspnoea and slight ruffled fur; these symptoms affected both male and female rats. Thereafter, all animals were free of symptoms. Body weight changes inconspicuous. Necropsy revealed no abnormalities.

Based on the results of the key study (A6.1.1) with SAS-HMDS in the CLH report, as well as the data included in the additional key elements section, the proposal for **no classification for acute oral toxicity** by the DS is supported by RAC.

Acute dermal toxicity

There was no study provided in the CLH report for acute dermal toxicity. However, in a study with SAS-DDS the LD_{50} was determined to be > 2000 mg/kg bw (Becker *et al.,* 2013). The DS noted that information from the skin irritation study with SAS-HMDS (Aerosil R812, A6.1.4) suggests a low dermal toxicity of the tested substance, since no mortality was observed at the dose of 0.5 g per animal. However, RAC believes this is a very low dose (about 200 mg/kg bw) to draw a conclusion. Nevertheless, the said study can be used as supporting evidence to the SAS-DDS study (Becker *et al*., 2013). Thus, based on the above, RAC concludes that **no classification for acute dermal toxicity is warranted**.

Acute inhalation toxicity

In the Tables below, the results from the two acute inhalation studies included in the CLH report are shown.

Table: Acute inhalation toxicity studies – CLH report

Species / Reference/ Year	Method	LC_{50} (mg/L)	Other observations
Wistar Rat / A6.1.3 / Degussa (Industry) 1983	GLP, No guideline method, reliability 2 (Klimisch) 5/sex/concentration One dose: 477 mg/m^3 The particle size distribution of the inhalable fraction revealed	> 0.48	No mortality observed Clinical results: During exposure, the animals were somewhat restless and their eyes were half-closed. Body weight decreased during the first 2 days of

	that about 56% of the particles had an aerodynamic diameter <5 µm (respirable). MMAD = 2.9 µm Whole body, 4 hour exposure SAS-DDS (Aerosil R974)		observations, but thereafter body weight gain turned back to normal. Necropsy: Pathology revealed no abnormalities.

The nominal concentration of the substance (SAS-DDS) in this study was calculated to be 24400 mg/m^3, while due to the design of the study (as mentioned in the CAR) the maximum attainable concentration was measured to be 477 mg/m^3. The difference between nominal and measured concentration (inhalable fraction) probably was related to the fact that, due to the electrostatic charge of the test substance particles, large amounts of test material were deposited on the walls and cage. Furthermore, the test substance mainly consisted of large aggregates with high settling speed under the influence of gravity. This experimental anomaly explains why only 2% of the total dust (nominal concentration) was the inhalable fraction (ratio analytical : nominal = ~500/~25000 x100) and why the maximum concentration attained was only 477 mg/m^3. At this specific dose there was no mortality, no pathological abnormalities and the clinical signs were not severe. Thus, the LC_{50} is estimated to be > 0.48 mg/L. RAC considers that this study does not provide adequate evidence for conclusion on classification to be drawn.

Table: Mechanistic study - Acute and long-term lung reaction following single intratracheal injection of SAS-HMDS

Species / Reference / Year	Method	Other observations
Wistar Rat / A6.10 / Degussa (Industry) 2005	No GLP, no guideline method Reliability 2 (Klimisch) 10/f/concentration Intratracheal application 0.15, 0.30, 0.60, 1.2 mg dust/lung Single administration with an observation period of 3, 21, and 90d Positive control rats were treated with 0.6 mg silica (quartz DQ12; particle diameter 0.9 µm) **SAS-HMDS (Aerosil R812S)**	No mortalities and no clinical signs were seen at the end of the 90 day observation. Following intratracheal instillation exposure, neither fibrogenic nor tumorigenic effects or chronic processes were observed at the concentrations tested. Symptoms indicative of inflammation in the deeper areas of the lung were reported at the start of the observation period, but were fully reversible by the end of the experiment. In contrast to the test substance, the examination of the positive control showed that the single injection of silica at 0.6 mg/lung induced an inflammatory reaction, which progressively became chronic; fibrosis was evident. A progressive cell proliferative reaction was evident.

The study focused on the possible lung-toxicity and DNA-damaging effect of SAS-HMDS following a single intratracheal injection in rats. During the first days after exposure, symptoms indicative of inflammation in the deeper areas of the lung were reported, as revealed by the increased number of cells, the increased rate of neutrophils and the increase in protein content in the rinsing solution (lavage). The degree of inflammation was clearly dose-dependent. The examinations after 21 days revealed that the inflammation process in the lung of the treated animals was reversible at all doses. No signs of fibrosis were evident. No signs indicating a progressive cell proliferative

reaction were seen.

In conclusion, although a short-term increased exposure by inhalation may induce acute inflammatory reactions in the lung, this effect is, however, reversible.

Literature studies

There are several studies with hydrophobic SAS as shown above that can be used for classification purposes in a weight of evidence approach.

The LC_{50s} in the studies presented in this opinion are summarised in the following Table. The results varied depending on the conditions of the experiment, down to the lowest value of 0.09 mg/L.

From the available studies it can be seen that surface area and particle size are factors that influence the outcome of the aforementioned studies. The test guidelines for acute inhalation toxicity with aerosols requires rodents to be exposed to an aerosol containing primarily respirable particles (with a MMAD of 1–4 µm), so that particles can reach all regions of the respiratory tract. For instance, solid materials are often micronised to a highly respirable form for testing, but in practice exposures will be to a dust of much lower respirability. In the case with the hydrophobic SAS, RAC believes that the intrinsic size of the substances is the nanoform and not the agglomerate, hence they are considered nanomaterials. RAC, nevertheless, acknowledges that these exposures may not necessarily reflect realistic conditions for SAS-HMDS and other hydrophobic SAS.

Table: Acute inhalation studies, LC_{50} values

Species / Reference / Year of the study$	**Substance**	**LC_{50} (mg/L)/ Classification****
BR Rat / ECETOC, 2006; Becker et al. 2013/ Cabot 1982 Study #1*	SAS-DDS (Aerosil R972, Degussa) Particle size/MMAD* 0.15 µm Exposure: 1h	> 2.28 No mortalities observed
Wistar rats/ ECETOC 2006, EPA 2011, Becker et al., 2013/ Cabot 1994 Study #2*	SAS-DDS, (Cab-O-Sil TS610) Particle size/MMAD*: 0.8-1 µm/1.175-1.275 µm Exposure: 4h	0.45 **Acute Tox. 2, H330**
Wistar rats / ECETOC, 2006 / Cabot 1994 Study #3*	SAS-HMDS (Cab-O-Sil TS530) Particle size/MMAD: 0.95-2.15 µm Exposure: 4h	0.09-0.84 Acute Tox. 2, H330 or Acute Tox. 3, H331
BR rats / Becker, 2013; EPA, 2011 / Cabot 2003 (revised) Study #4*	SAS-DDS Particle size/MMAD: 1.24 µm Exposure: 4h	0.52-1.12 Acute Tox. 3, H331 or Acute Tox. 4, H332
SD rats / ECETOC, 2006 / Wacker 1996 Study #5*	SAS-HMDS, HDK SKS130 Particle size/MMAD: < 0.2 µm Exposure: 4h	1.65 Acute Tox. 4, H332

SD rats / ECETOC, 2006 / Wacker 1996 Study #6*	SAS-DDS, HDH SKS130 Particle size/MMAD: 7.2-7.7 µm Exposure: 4h	> 2.2 (40% mortality)
SD rats / ECETOC, 2006 / Wacker 1996#	SAS-HMDS***, HDK SKS 300 Particle size/MMAD < 0.1 µm Exposure: 4h	0.09 **Acute Tox. 2, H330**
SD rats / ECETOC, 2006 / Wacker 1996#	SAS-HMDS***, HDK SKS 300 Particle size/MMAD = 7.0-7.1 µm Exposure: 4h	0.5 **Acute Tox. 2, H330**
Wistar Rat / A6.1.3 / Degussa 1983	SAS-DDS (Aerosil R974) Particle size/MMAD = 2.9 µm Exposure: 4h	> 0.48

$ The references are to review articles where the studies are mentioned, as well as the source and year of the actual study

* Refer to Table "Acute inhalation toxicity studies with all three forms of hydrophobic SAS available in the open literature" (in the Background Document) for further detail

** Refer to values for dusts and mists in Table 3.1.1 of Annex I of the CLP Regulation

*** Becker et al. (2013) provides particle size dimensions in µm; ECETOC (2006) provides particle size/MMAD (calculated by Cascade impactor) in µm; MMAD is defined as the aerodynamic diameter at which 50% of the particles by mass are larger and 50% are smaller

No details apart from the LC_{50} are provided

The available studies clearly show that hydrophobic SAS (all three forms discussed in this document) have an acute inhalation effect in the rat. As seen in the following Table, experimental LC_{50} values point to a classification for acute toxicity via inhalation between categories 2 and 3. Study #2 (below) was an acute inhalation toxicity study with one of the relevant forms of hydrophobic SAS available in the open literature (SAS-DDS – Cab-O-Sil TS610). The conditions of the study were according to OECD TG 403, regarding MMAD, exposure type and period and observation time, and gave an LC_{50} of 0.45 mg/L, and this study can be considered to be a key study for the purposes of classification and for establishing an ATE (although it is acknowledged to be conservative). The LC_{50} of 0.45 mg/L was also quoted in the EPA evaluation for SAS-DDS (EPA, 2011).

Therefore, RAC does not support the DS opinion for no classification and proposes to **classify Silanamine as Acute Tox. 2, H330**. Additionally, RAC proposes **an ATE value of 0.45 mg/L**.

10.4 Skin corrosion/irritation

Table 10.4.1: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
OECD 404 (1981), GLP	New Zealand white Rabbit 3 m, 3 f	Aerosil R 812	(0.5 g) in polypropylene glycol/water (1:1) 4 hours of exposure on an intact (9cm^2) and an abraded (6.25 cm^2) shaved dorsal skin area.	No erythema was seen, neither on intact nor on abraded skin. The mean score for erythema was 0. No edema was seen, neither on intact nor on abraded skin. The mean score for edema was 0. Neither mortality nor symptoms of toxicity were seen. Body weight gain was inconspicuous. No test substance-related skin discoloration was seen within the application areas.	IIIA6.1.4

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

Rabbits received Aerosil R812 moistened with polypropylene glycol and physiological saline (1:1) on intact and abraded skin in a study performed according to OECD guideline 404.
No skin reaction was observed.

10.4.2 Comparison with the CLP criteria

Scores for erythema and edema are 0.
This score value is out of the range for classification under regulation (EC) 1272/2008.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Based on the results of the skin irritation study, no classification is proposed for the active substance.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The skin corrosion/irritation potential of silanamine (SAS-HMDS, Aerosil R812) has been investigated in one *in vivo* study in the rabbit. The DS proposed no classification based on the OECD TG404 and GLP compliant study A6.1.4.

Comments received during public consultation

No comments were received regarding the skin corrosion/irritation properties of silanamine.

Assessment and comparison with the classification criteria

Table: Skin irritation study in the CLH report

Type of study/ Reference / Year	Method Test substance	Dose levels Exposure	Observations
In vivo NZW rabbit / A6.1.4 / 1984	OECD TG 404 GLP Reliability: 1 3/sex 4-hour exposure 72 hours observation period Silanamine (SAS-HMDS)	0.5 g in polypropylene glycol/water (1:1) 4 hours of exposure on an intact (9 cm^2) and an abraded (6.25 cm^2) shaved dorsal skin area.	All animals survived the test and were free of symptoms. Body weight gain was similar for all animals and inconspicuous. Neither erythema nor oedema were observed on the intact or abraded skin. No test substance-related skin discoloration was seen.

Data from literature reviews (Becker *et al.,* 2013; EPA, 2011; ECETOC, 2006) confirmed the lack of skin irritation properties for all three hydrophobic SAS, as no signs of irritation were observed in several studies. Application of various SAS to the skin of rabbits for up to 24 hours generally produced no signs of irritation. Occasionally, very slight erythema (primary irritation index 0.25 - 0.44 out of 8 maximum) has been reported; such effects were rapidly reversible.

Thus, based on the results from the A6.1.4 study and the data from the literature review, RAC agrees with the DS that **no classification is warranted for skin corrosion/irritation.**

10.5 Serious eye damage/eye irritation

Table 10.5.18: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
OECD 405 (1981), GLP	New Zealand white Rabbit 3 m, 3 f	Aerosil R 812	The test substance was applied undiluted 0.1 g First group of animals	Examination time points: 60min, 24h, 48h and 72h following instillation of the test substance. Neither mortality nor symptoms of toxicity were seen. The body weight gain	IIIA6.1.4

			(3 males): eyes remained untreated following instillation of the test substance. Second group of animals (3 females): eyes were rinsed for one minute with physiological saline after 30 seconds following the instillation of the test substance.	was similar for all animals and inconspicuous. For both, rinsed and non-rinsed treated eyes, no corneal opacity was seen; an average score of 0 was reported for all examination time points and all animals. For both, rinsed and non-rinsed treated eyes, the iris remained inconspicuous; an average score of 0 was reported for all examination time points and all animals. At examination time point 60 minutes, all animals with non-rinsed treated eyes as well as one animal of the "rinsed"-group displayed redness of the conjunctiva (scored 1 for each animal). This effect disappeared in all concerned animals within 24 hours, indicating reversibility. At all further examination time pints (24, 48 and 72h), no more redness of the conjunctiva was seen (score = 0). No chemosis was seen. No test substance-related discoloration of the cornea and/or conjunctiva was seen.	

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

10.5.2 Comparison with the CLP criteria

After instillation of 0.1g of Aerosil R812 in rabbits, an irritation score of 0.25 was reported when the treated eyes were not rinsed. Following rinsing, the irritation score was lowered to 0.08. These slight signs of irritation were reversible and disappeared within 24 hours.

These score values are out of the range for classification under regulation (EC) 1272/2008.

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Based on the results of the eye irritation study, no classification is proposed for the active substance.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The eye damage/irritation potential of silanamine (SAS-HMDS) has been investigated in one *in vivo* study in the rabbit. The DS proposed no classification based on the OECD TG 405 and GLP compliant study A6.1.4.

Comments received during public consultation

No comments were received regarding the eye damage/irritation properties of silanamine.

Assessment and comparison with the classification criteria

Table: Eye damage/irritation study in the CLH report

Type of study/ Reference / Year	**Method Test substance**	**Dose levels Exposure**	**Observations**
In vivo NZW rabbit / A6.1.4 / 1984	OECD TG 405 GLP Reliability: 1 3 males and 3 females 4-hour exposure 72 hours observation period SAS-HMDS (Aerosil R812)	The test substance was applied undiluted in the left eye of each animal; the application amount was 0.1 g. The right eye remained untreated and served for control. For all males, the treated eyes were not rinsed, whereas for all females, the treated eyes were rinsed about 30 seconds following instillation of the test substance. The eyes were examined for signs of irritation affecting the cornea, the iris and the conjunctiva at following time points: 60 min, 24h, 48h and 72h following application. Assessment of the findings was based on guideline.	All animals survived the test and were free of symptoms. Body weight gain was similar for all animals and inconspicuous. At examination time point 60 minutes, all animals with non-rinsed treated eyes, as well as one animal of the "rinsed"-group displayed redness of the conjunctiva (scored 1 for each animal). This effect disappeared in all concerned animals within 24 hours (reversible). At all further examination time points (24, 48 and 72h), no more redness of the conjunctiva was seen (score= 0). No chemosis affecting the conjunctiva was seen and both, the cornea and the iris were inconspicuous. No test substance-related discoloration of the eye was seen.

Data from a literature review (Becker *et al*., 2013; EPA, 2011; ECETOC, 2006) corroborated that none of the three hydrophobic SAS had eye irritation properties. Instillation of various SAS (hydrophobic, pyrogenic surface treated silica) into the rabbit eye resulted in no or slight irritation (slight erythema); the effect was completely and rapidly reversible. After washing the eyes, no irritation was observed.

Slight signs of irritation were seen in the key study of the CLH report with SAS-HMDS as well as in the literature studies with all three forms of hydrophobic silica referenced in this opinion. In addition, chromodacryorrhea and blepharospasm were observed in one acute inhalation study at a single dose. In all cases, the signs were reversible and disappeared within 24 hours. Thus, based on the results from the key A6.1.4 study and the data from the literature review, RAC agrees with the DS that **no classification is warranted for eye damage/irritation.**

10.6 Respiratory sensitisation

No data on the potential of pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide to induce respiratory sensitisation are available.

10.7 Skin sensitisation

Table 10.7.1: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results**	**Reference**
OECD 406 (1981), GLP (Maurer optimization test)	Dunkin-Hartley albino Guinea pigs 24 m and 24 f	Aerosil R 812 A separate test was conducted with di-nitro-chloro benzene and was positive.	Induction: 0.1% Challenge: 0.1 % (first challenge) and 30% (second challenge)	At 24h: negative control: 0/24 treated: 0/24 At 48h: negative control: 0/24 treated: 0/24 Neither mortality nor clinical symptoms of toxicity were reported. The animals were inconspicuous and their body weight gains were not affected by the experiment.	IIIA6.1.5

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

For induction, 0.1 ml of a 0.1% dilution of test substance in physiological saline and propylene glycol (1:1) has been applied.
A first challenge was conducted 13 days after the last treatment of the third induction. A second challenge was conducted 13 days after the first one.
For the first challenge, 0.1 ml of a 0.1% dilution of test substance in physiological saline and propylene glycol (1:1) has been used.
For the second challenge; 30% of test substance in Vaseline has been applied.

None of the treated animals showed a positive reaction 24h and 48h after challenge.
Neither mortality nor clinical symptoms of toxicity were reported. All animals were inconspicuous and their body weight gains were not affected by the experiment.

10.7.2 Comparison with the CLP criteria

None of the treated animals showed a positive reaction 24h and 48h after challenge. Thus, no classification under regulation (EC) 1272/2008 is required.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Based on the results of the skin sensitization study, no classification is proposed for the active substance.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The skin sensitisation potential of silanamine was investigated in one Guinea Pig Maximisation Test (Maurer Optimisation Test). The DS proposed no classification based on the OECD TG 406 and GLP compliant study A6.1.5.

Comments received during public consultation

No comments were received regarding skin sensitisation.

Assessment and comparison with the classification criteria

Table: Skin Sensitisation study in the CLH report

Type of study / Reference / Year	Method Test substance	Dose levels Exposure	Observations
GPMT Dunkin-Hartley albino	OECD TG 406 GLP Reliability: 1	Induction: The test substance was applied as a 0.1% dilution in physiological saline and propylene glycol (1:1) for all 3	None of the treated animals showed a positive reaction. Neither mortality nor clinical symptoms of toxicity were

Guinea pigs / A6.1.5 / 1984	24/sex Silanamine (SAS-HMDS) A separate test was conducted with di-nitro-chloro benzene and was positive	weeks of induction. However, for week two and three, the 0.1% dilution further was mixed 1:1 with Freund's adjuvant. Challenge: For the first challenge, the tests substance was applied as 0.1% dilution in physiological saline and propylene glycol (1:1). For the second challenge, the test substance was applied as a 30% mixture in vaseline.	reported. All animals were inconspicuous and their body weight gains were not affected by the experiment. None of the negative control animals showed a positive reaction whereas all animals of the DNCB control group reacted positively.

Note: The guinea pig tests should be conducted at the highest induction dose causing mild (Buehler Assay) or mild-to-moderate (GPMT) skin irritation. No such data were available for this study.

There were no animal studies in the open literature for skin sensitisation for SAS-HMDS and the two read across SAS. However, there have been no cases of sensitisation in humans reported in decades of manufacture and use of all forms of SAS (information from producers, Pölloth *et al.,* 2012). In addition, SAS-DDS up to 30% as a pure substance or up to 7% as an ingredient in cosmetic products was not sensitising in multiple human repeat insult patch tests (Becker *et al.*, 2013). Furthermore, the chemical composition/structure of all three forms of surface treated SAS used in this opinion do not indicate any sensitising potential.

Thus, based on the results from the A6.1.5 study, on the negative results of the HRIPTs with the read across SAS-DDS (Becker *et al*., 2013), on the fact that there have been no cases of sensitisation in humans reported in decades of manufacture and use (Pölloth *et al*., 2012) and, since the chemical composition of surface treated SAS-HMDS does not indicate a sensitising potential, RAC agrees with the DS that **no classification is warranted for skin sensitisation.**

10.8 Germ cell mutagenicity

Table 10.8.1: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
Similar to OECD 471 and 472, no GLP	Toluene-extract of Aerosil R 812 (values related to original amount of Aerosil).	*S. typhimurium*: TA 1537, TA 98, TA 100 *E. coli*: WP2 uvr A 0, 5, 15.8, 50, 158, 500, 1 580 and 5 000 µg/plate	Slight cytotoxicity from 1 580 to 5 000 µg/plate in the absence of S9 mix. The results for the negative and positive control plates were as expected. **Results:** Negative with S9 mix	IIIA6.6.1

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
			Negative without S9 mix	
Guideline-like, acc. to Evans 1976, no GLP	Tested silica: Cab-O-Sil TS-610 [CAS 68611-44-9] (amorphous, surface-treated silica)	Chinese hamster Ovary (CHO) cells 0, 63, 125, 250, and 500 µg/ml	The test article was insoluble in the solvent (DMSO) at a stock concentration of 50 mg/ml and insoluble in treatment medium at a concentration of 500 µg/ml, it was soluble at all other concentrations tested. Cytotoxicity observed at 500 µg/ml: ~37 % (-S9) ~28 % (+S9) The positive and negative controls fulfilled the requirements for a valid test. **Results:** Negative with S9 mix Negative without S9 mix	IIIA6.6.2
OECD 476, GLP	Aerosil R 812 S	Mouse Lymphoma L5178Y cells (TK+/-) 0, 2.34, 4.69, 9.38, 18.8, 37.5 µg/ml (+/- S9 mix), and 150 µg/ml (-S9 mix)	Precipitation: ≥ 37.5 µg/ml (± S9 mix) Cytotoxicity: In the main tests, no significant impact on relative survival was noted in any test combination. Expected results were obtained with solvent and positive controls. **Results:** Negative with S9 mix Negative without S9 mix	IIIA6.6.3

Table 10.7.2: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Bronchiolar Lavage: Radical-induced 8-OH-Guanine	Aerosil R 812 S	Rat Wistar 5 f/group Administration route: intra-tracheal (single exposure)	Increase in 8-OH-G, reversible but no clear dose-response relationship - 3 days after exposure: At the application dose of 0.15 and 0.30 mg/lung, the content of 8-OH-	IIIA.6.6.4

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
(8-OH-G) content in alveolar cells no GLP		0, 0.15, 0.30, 0.60, and 1.2 mg dust/lung Sampling times: 3 d, 21 d, and 90 d after the single exposure	G radical-induced in alveolar cells is ~2x control; At the application dose of 1.2 mg/lung, the content of 8-OH-G radical-induced in alveolar cells is ~3x control; - 21 days after the exposure: A slight increase of the content of 8-OH-G radical-induced in alveolar cells (< 2 x control) is observed at the application dose of 1.2 mg/lung. - 90 days after the exposure: No increase of the content of 8-OH-G radical-induced in alveolar cells is observed.	
Bronchiolar Lavage: Mutated p53 protein no GLP	Aerosil R 812 S	Rat Wistar 5 f/group Administration route: intra-tracheal (single exposure) 0, 0.15, 0.30, 0.60, and 1.2 mg dust/lung Sampling times: 3 d, 21 d, and 90 d after the single exposure	No mutated p53 protein was detected by monoclonal antibodies raised against a specific epitope of the protein.	IIIA.6.6.4

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

In vitro studies

Pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide (Aerosil R 812, Cab-O-Sil TS-610) were tested in *in vitro* tests.

In an Ames test (Doc IIIA 6.6.1), Aerosil R 812, negative results are observed in *S. typhimurium* TA 1537, TA 98, TA 100 and E. coli WP2 uvr A at doses up to 5 000 µg/plate with and without S9 mix. Nevertheless, several deficiencies were noted in this study such as only 4 strains used and product tested as a toluene extract (only liposoluble fraction was therefore analysed) without data on the solubility in this solvent. At the highest dose, the extract formed

a macroscopically visible precipitate on the test plates that was still present at the end of the experiment. The cytotoxicity test revealed a weak toxicity at the two highest tested concentrations of the toluene extract from Aerosil R 812 (i.e. at 1580 and 5000 µg/plate), in the absence of S9 mix. An increase of number of revertant colonies was reported only at the highest tested dose with TA 100, in the presence of S9 mix (weak equivocal mutagenic effect).

A chromosome aberration test was performed with Cab-O-Sil TS-610, a synthetic pyrogenic amorphous surface-treated silica, in CHO cells (Doc IIIA 6.6.2) and gave negative results. Nevertheless, deviations from the guideline were noted, such as the lack of a second continuous experiment without S9 mix, since the first experiment gave negative results and the number of analyzed cells was half the OECD recommendations.

An *in vitro* gene mutation assay in mouse lymphoma L5178Y cells (TK+/-) run in compliance with the guideline OECD 476 (Doc IIIA 6.6.3) has been performed with Aerosil R 812S in order to cover the detection of gene mutations and chromosome aberrations.
In the absence of S9 mix, there was no evidence of mutagenic effect.
In the presence of S9 mix, there was no evidence of mutagenic effect even if in the second assay, a positive linear trend was present. However, the individual values were included in the range of values for the negative control and no statistically significant increases in mutant frequency were observed. Moreover, the IMF (induced mutation frequency) was lower than the GEF (global evaluation factor) and in the first experiment, this trend was not significant.

***In vivo* studies**

A mechanistic *in vivo* assay (Doc IIIA.6.6.4) has been provided and considered as supportive document. The study focused on observed lung damages and markers of toxicity after exposure of rats to amorphous Aerosil R 812 S, compared to the positive lung carcinogen, a crystalline silica (quartz) dust.

Aerosil R 812 S was given by a single intra-tracheal injection to rats and followed by a 90 day post-exposure period. The Aerosil R 812 S data were compared to the effect of a crystalline silica (quartz) dust which is a known toxic to lungs and carcinogenic. This test followed no guideline and was not conducted according to GLP. Four different parameters were evaluated in this mechanistic study: the measurement of DNA-adducts (8-OH-guanine), markers of inflammation, histological analysis and presence of mutant p53.

Concerning the mutagenicity issue, it was found that, following treatment with Aerosil R 812 S, the 8-OH-guanine level increased significantly in DNA during the first period of the post-exposure phase, and was not persistent thereafter, returned to background level after 90 days of recovery while the signs of acute inflammation were also decreasing.
On the contrary, the crystalline silica (quartz) induced a high and persistent reaction (although the increase of 8-OH guanine level was below the values after exposure to Aerosil R 812 S, this reaction persists over the time). These increases of primary DNA lesions could be explained by an inflammation response which was associated with the production of reactive oxygen species (ROS).
Additionally, the Ab-1 mutant-specific (Epitope aa 212-217) mouse monoclonal antibodies failed to detect the presence of mutant tumour suppressor protein p53 after exposure to Aerosil R 812 S, while the crystalline silica (quartz) caused a significant accumulation of mutated p53 protein over time.

10.8.2 Comparison with the CLP criteria

Negative results are observed in *in vitro* and *in vivo* tests with and without metabolic activation.

The criteria for classification as mutagenic under regulation (EC) 1272/2008 are not fulfilled.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Based on the results of the *in vitro* and *in vivo* mutagenicity tests, no classification is proposed for the active substance.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification based on negative results in the following *in vitro* and *in vivo* assays:

- Bacterial reverse mutation test, Ames test (A6.6.1)
- *In vitro* mammalian chromosome aberration test (A6.6.2)
- *In vitro* mammalian cell gene mutation test (A6.6.3)
- *In vivo* genotoxicity and gene mutation assay (A6.6.4)

Comments received during public consultation

Comment 1

An MSCA stated that an increase in 8-OH-guanine DNA adducts was observed in lung cells after intratracheal installation. Even though this change may be only temporarily, it is a change of the structure of the DNA. Therefore, it fulfils the definition for genotoxicity (CLP Regulation, Annex I, 3.5.1.3). Thus, it cannot be concluded that all studies were negative. However, an increase in genotoxicity in somatic cells in the absence of positive mutagenicity tests *in vivo* or *in vitro* is insufficient for classification.

Comment 2

An MSCA emphasized that the data do not enable a conclusion to be drawn on the mutagenic potential of SAS-HMDS based on the available data. The MSCA preferred that it be stated in the RAC opinion that classification is not warranted due to insufficient data.

Additional key elements

In following Tables data from several *in vitro* studies with various forms of hydrophobic SAS are shown. In a series of Ames tests, hydrophobic SAS were not gene point mutagens *in vitro*, using

Salmonella typhimurium and *Escherichia coli* bacteria and did not induce chromosomal aberrations in Chinese hamster ovary cultured mammalian cells (CHO). More details on the studies and the actual raw data were not available.

***Table**: In vitro mutagenicity studies in micro-organisms from the literature (ECETOC, 2006; Becker et al., 2013)*

Type of SAS / Product Name	Test System	Metabolic Activation	Concentration (µg/plate)	Cytotoxicity	Result
SAS-HMDS / Cab-O-Sil TS500	*S. typhimurium*, TA98, TA100, TA1537	± S9	1580	None	Negative
SAS-HMDS / Cab-O-Sil TS530	*S. typhimurium*, TA98, TA100, TA 1535, TA1537, TA1538	± S9	5000	None	Negative
SAS-DDS / Cab-O-Sil TS610	*S. typhimurium*, TA98, TA100, TA 1535, TA1537, TA1538	± S9	5000	None	Negative
SAS-PDMS / Cab-O-Sil TS720	*S. typhimurium*, TA98, TA100, TA 1535, TA1537, TA1538	± S9	5000	None	Negative
SAS-PDMS / HDK H2015EP	*S. typhimurium*, TA98, TA100, TA 1535, TA1537, E coli WP2	± S9	5000	None	Negative
SAS-PDMS / HDK H2050EP	*S. typhimurium*, TA98, TA100, TA 1535, TA1537, *E. coli WP2*	± S9	5000	None	Negative

***Table:** In vitro mutagenicity an chromosomal aberration studies in Chinese hamster ovary (CHO) mammalian cells from the literature (ECETOC, 2006; Becker et al., 2013)*

Type of SAS / Product Name	Test System	Metabolic Activation	Concentration (µg/ml)	Cytotoxicity Result
SAS-HMDS / Cab-O-Sil TS500	CHO	± S9	63-500	No clastogenic activity
SAS-HMDS / Cab-O-Sil TS530	CHO	± S9	63-500	No clastogenic activity
SAS-DDS / Cab-O-Sil TS610	CHO	± S9	63-500	No clastogenic activity

SAS-PDMS / Cab-O-Sil TS720	CHO	± S9	42-333	No clastogenic activity

There were no *in vivo* studies with hydrophobic SAS in the open literature.

Assessment and comparison with the classification criteria

Table: In vitro *mutagenicity studies in the CLH report*

Type of study / Reference / Year	Method Test substance	Observations
Bacterial reverse mutation test (Ames test) / A6.6.1 / 1983	*OECD TG 471/472 (similar)* *No GLP* Reliability: 2 *S. typhimurium* TA 1537, TA 98, TA 100 *E. coli*: WP2 uvr A 0, 5, 15.8, 50, 158, 500, 1 580 and 5 000 µg/plate SAS-HMDS (Aerosil R812)	Cytotoxicity test: Slight cytotoxicity from 1580 to 5000 µg/plate in the absence of S9 mix. Negative and positive controls: The results for the negative and positive control plates were as expected. Results: no mutagenicity was observed in the absence of S9 mix. In contrast, in the presence of S9 mix, a dose-related (r^2=0.99) increase in the number of revertant colonies was reported for the *S. typhimurium* strains, especially for TA 100; however, only at 5000 µg/plate doubling of the number of colonies was observed. In total, the effect was very weak. In conclusion: **Negative or weak response with S9 mix** **Negative without S9 mix**
In vitro mammalian chromosome aberration test / A6.6.2 / 1995	*Standard procedures of Evans (1976), Guideline-like (similar to OECD 473)* *GLP* Reliability: 1 0, 63, 125, 250, and 500 µg/mL **SAS-DDS** **(Cab-O-Sil TS-610)** CAS 68611-44-9 belongs to the Aerosil series R972, R974, R976, corresponding best to **Aerosil R972**	The test article was insoluble in the solvent (DMSO) at a stock concentration of 50 mg/mL and insoluble in treatment medium at a concentration of 500 µg/mL, it was soluble at all other concentrations tested. Cytotoxicity observed at 500 µg/mL: ~37% (-S9) ~28% (+S9) The positive and negative controls fulfilled the requirements for a valid test. Results: Neither in the presence nor in the absence of S9 mix, there was a significant increase in the percentage of treated cells with structural aberrations. Criteria for validation of the test: The frequency of cells with structural chromosome aberrations in either the untreated or the solvent control must not exceed 6%. The frequency of cells

		with structural chromosome aberrations in the positive controls must be statistically increased ($p \leq 0.05$, Fisher's exact test) relative to untreated or solvent control. Conclusions: **Negative with S9 mix** **Negative without S9 mix**
In vitro mammalian cell gene mutation test / A6.6.3 / 2008	OECD 476 GLP Reliability: 1 Mouse Lymphoma L5178Y cells (TK+/-) 0, 2.34, 4.69, 9.38, 18.8, 37.5 µg/mL (+/- S9 mix), and 150 µg/mL (-S9 mix) SAS-HMDS (Aerosil R812S)	Precipitation: ≥ 37.5 µg/ml (± S9 mix) Cytotoxicity: In the main tests, no significant impact on relative survival was noted in any test combination. Expected results were obtained with solvent and positive controls. Results: In the absence of S9, there was no evidence of a mutagenic effect in both experiments, after 3h and 24h exposure. In the presence of S9, there was no evidence of a mutagenic effect in both experiment after 3h exposure. In the second experiment, a linear trend was indicated. Since no statistically significant increases in mutant frequency were observed, the apparently linear trend was considered to be attributable to a chance event, not related to the action of the test substance and of no biological significance. Conclusion SAS-HMDS (Aerosil R812S does not induce mutation at the TK locus of L5178Y mouse lymphoma cells in vitro in the absence or presence of S9 metabolic activation, under the reported experimental conditions.

Table: In vivo *studies in the CLH report*

In vivo genotoxicity and gene mutation assay / A6.6.4 / 2005	No guideline No GLP Reliability: 2 Wistar rat 10 females per group Administration: Single intratracheal injection Dose: 0.15, 0.30, 0.60, 1.2 mg dust/lung	8-Oxoguanine contents in lung cells: The DNA-examination in the lung cells for 8-oxoguanine content revealed increased amounts following the treatment (3 day post-exposure) when compared to the negative control; no clear dose-response relationship was evident at this time point. For the positive controls treated with silica (quartz DQ12; particle diameter 0.9 µm) 8-oxoguanine contents also were

	Sampling time: 3d, 21d and 90d SAS-HMDS (Aerosil R812S)	increased, but were below the values obtained for SAS-HMDS. After 21 days, the 8-oxoguanine contents for the SAS-HMDS treated animals nearly returned to negative control values; in fact at this time point, especially at the higher doses, significant differences from controls were still evident. After 90 days, all measured 8-oxoguanine levels in SAS-HMDS treated animals returned to control values; in contrast, animals treated with the positive control silica (quartz DQ12; particle diameter 0.9 µm) still showed significantly increased amounts of 8-oxoguanine in their lung cells. p53: No p53 (mutant)-positive cells could be found for the SAS-HMDS treated animals; in contrast, positive controls (quartz) showed a significant increase in positive cells (21 and 90 days).

In vitro

A6.6.1: There were several deficiencies noted in the Ames test with SAS-HMDS, including that only four instead of the minimum five strains recommended in the OECD TG 471 were used and that the product was tested as a toluene extract (only the liposoluble fraction was therefore analysed) without data on the solubility in this solvent. A weak mutagenic effect was reported in presence of S9 mix especially for the *S. typhimurium* TA 100 strain at the highest test concentrations. According to Ames *et al*. (1975), a compound is considered negative if it was tested up to 500 µg/plate and did not double the number of colonies above control. This criterion was fulfilled as a doubling of the number of revertant colonies was seen only at the highest tested dose of 5000 µg/plate. Therefore, the tested toluene extract of SAS-HMDS can be considered as non-mutagenic. In addition, surprisingly, the DMSO concentration increased with the dose. Moreover, according to Elespuru *et al*. (2018), the *S. typhimurium* and *E. coli* strains do not take up or respond to nanomaterials and as a result it is recommended to use data from an *in vitro* mammalian mutagenicity assay instead of a bacterial mutation test. Results from negative bacterial assays are not definitive as a test result for nanomaterials.

A6.6.2: This is a literature study (similar to OECD TG 473) included in the CLH report with SAS-DDS(Cab-O-Sil TS-610). There were deficiencies in this study, the more notable being the number of analysed cells (100 instead of the recommended 300 cells/concentration). SAS-DDS was negative in the *in vitro* chromosome aberrations assay conducted with CHO cells. The positive and negative controls fulfilled the requirements for a valid test.

A6.6.3: An *in vitro* gene mutation assay in mouse lymphoma L5178Y cells (TK+/-) was performed with SAS-HMDS (Aerosil R812S) in order to cover the detection of gene mutations and chromosome aberrations (OECD TG 476). In the study, no mutagenicity was observed in the absence of S9 mix. In contrast, in the presence of S9 mix, in the second assay, a positive linear

trend was present. However, the individual values were included in the range of values for the negative control and no statistically significant increases in mutant frequency were observed. Moreover, the induced mutation frequency was lower than the global evaluation factor and in the first experiment, this trend was not significant. Thus, SAS-HMDS does not induce mutation at the TK locus of L5178Y mouse lymphoma cells *in vitro* in the absence or presence of S9 metabolic activation.

In conclusion, the chromosomal aberration and the gene mutation assays from the open literature, as well as from the CLH report demonstrate that SAS-HMDS did not induce gene mutations in CHO cells or chromosomal aberrations in cultured mammalian cells. In addition, the hydrophobic SAS are not point mutagens *in vitro*, using *Salmonella typhimurium* and *Escherichia coli*, although the latter studies are not recommended for nanomaterials.

In vivo

The A6.6.4 *in vivo* mechanistic study focused on observations on lung damage and markers of toxicity after exposure of rats to SAS-HMDS (Aerosil R812S) and compared the data with known positive lung carcinogen crystalline silica (quartz) dust. SAS-HMDS was given by a single intratracheal injection to rats and followed by a 90 days post-exposure period. The SAS-HMDS data were compared to the effect of a crystalline silica (quartz) dust which is known to be toxic to lungs and carcinogenic. This test followed no guideline and was not conducted according to GLP. Four different parameters were evaluated in this mechanistic study: the measurement of DNA adducts (8-OH-guanine), markers of inflammation, histological analysis and presence of mutant p53 gene.

8-Oxoguanine contents in lung cells

The DNA-examination in the lung cells for 8-oxoguanine content revealed increased amounts following the treatment (3 days post-exposure) when compared to the negative control; no clear dose-response relationship was evident at this time point. For the positive controls treated with silica (quartz DQ12; particle diameter 0.9 µm) 8-oxoguanine contents also were increased, but were below the values obtained for SAS-HMDS. After 21 days, the 8-oxoguanine contents for the SAS-HMDS treated animals nearly returned to negative control values; in fact at this time point, especially at the higher doses, significant differences from controls were still evident. After 90 days, all measured 8-oxoguanine amounts in SAS-HMDS treated animals returned to control values; in contrast animals treated with the positive control silica (quartz DQ12; particle diameter 0.9 µm) still showed significantly increased amounts of 8-oxoguanine in their lung cells.

The increase in 8-oxoguanine content in the lungs is the result of a structural change of the DNA and shows that SAS-HMDS could have mutagenic potential. However, when that change is fully reversible it indicates that 8-oxoguanine is fully restored, probably reflecting accurate base excision repair or translesion synthesis without mutation, which is the case for silanamine. In contrast, Yasui *et al.* (2014), examined the fate of the nucleoside of 8-oxoguanine, 8-oxo-dG, when this oxidised derivative of deoxyguanosine was inserted into the thymidine kinase gene in a chromosome within human lymphoblastoid cells in culture. They inserted 8-oxo-dG into about 800 cells, and could detect the products that occurred after the insertion of this altered base, as determined from the clones produced after growth of the cells. 8-Oxo-dG was restored to guanine (G) in 86% of the clones, probably reflecting accurate base excision repair or translesion synthesis without mutation. G:C to T:A transversions occurred in 5.9% of the clones, single base deletions in 2.1% and G:C to C:G transversions in 1.2%. Together, these more common mutations totalled 9.2% of the 14% of mutations generated at the site of the 8-oxo-dG insertion. Among the other

mutations in the 800 clones analysed, there were also 3 larger deletions, of sizes 6, 33 and 135 base pairs. Thus 8-oxo-dG, if not repaired, can directly cause frequent mutations, some of which may contribute to carcinogenesis. In addition, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and 8-oxo 7,8-dihydroguanosine (8-oxoG) have been commonly chosen as the biomarkers of oxidative damage to DNA and RNA, respectively and shown to be over-expressed in patients compared with controls in different types of cancers, neurodegenerative disorders and chronic diseases (Guo *et al.,* 2017*)*.

In conclusion, although in the study with SAS-HMDS the increase of 8-oxoguanine content in the lungs was fully reversible, it cannot be excluded that chronic exposure to high level of silanamine could lead to a saturation of the DNA repair mechanism and give rise to mutations.

On the other hand, in the same study (A6.6.4), the Ab-1 mutant-specific (Epitope aa 212-217) mouse monoclonal antibodies failed to detect the presence of mutant tumour suppressor protein p53 after exposure to SAS-HMDS, while the crystalline silica (quartz) caused a significant accumulation of mutated p53 protein over time, thus providing evidence that no mutation was produced in the DNA from exposure to silanamine. However, it should be noted that the fact that the Ab-1 antibody does not detect mutant p53 does not ensure that no mutation is induced in the cell. Furthermore, the transient increase in DNA damage reflected by an increase in 8-oxoguanine in the DNA could be explained by the acute inflammation response, which is associated with the transiently enhanced formation of oxygen radicals instead of mutagenic effects as exemplified by the genetic analysis of the p53 locus. It is stated both in the CAR and the CLH report that inflammation markers were monitored in the study but details were not given.

In conclusion, there is a series of *in vitro* tests (gene mutation test in bacteria, chromosomal aberration test and mouse lymphoma assay ($tk^{+/-}$ locus) from the literature and the CLH report which are all negative, although some of them had deficiencies (especially the bacteria tests). There is also an *in vivo* mechanistic study with equivocal results, which could indicate mutagenic properties for SAS-HMDS and there is no *in vivo* test in somatic cells to complete the required testing for mutagenicity (CLP Regulation). Therefore, RAC considers that studies necessary for a scientifically sound evaluation of the mutagenic properties of SAS-HMDS are missing, and thus **proposes no classification for mutagenicity due to insufficient/ inconclusive data**

10.9 Carcinogenicity

Table 10.9.1: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Oral, feed no guideline study Rat Wistar 20 m,	Aerosil R 972 100 mg/kg bw/d	At the end of the experimental period, the treated males weighed between 275 and 490. The treated females weighed between 205 and 445 g. Body weight increase was within the range of normal untreated male rats	IIIA6.7

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
20 f The test substance was offered to the animals in feed balls. The animals received these balls in the morning prior to getting any other feed; particular attention was given to complete intake of these balls containing the test substance. The controls were fed with the same feed as treated animals but without test substance. The animals were examined for general state of health and clinical symptoms of toxicity.	7 d/wk 24 months	(control) and was therefore inconspicuous. No clinical effects was observed in males and females. One male displaying a visible tumor (benign fibrosis adenoma). In the animals showing signs of chronic bronchopneumonia, haematology revealed hyperleukocytosis with polynucleosis as well as hypergammaglobulinemia. The remaining animals showed no abnormalities and normal electrophoresis-values. No treatment-related development of tumor was observed. No subcutaneous sarcoma, no pituitary gland tumors and no tumors in testes were seen. A benign tumor (fibro–adenoma) was seen in one male; such tumors also occur in control Wistar rats. No signs of leucosis were seen.	

Table 10.9.2: Summary table of human data on carcinogenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Health survey (five German plants): 497 exposed workers, 206 not exposed Investigations performed between 1995 and 2000	Specificity of the substance not known (surface-treated or not)	Concentration unknown Chronically exposed (duration unknown)	No tumours. No evidence of long-term pulmonary effects.	IIIA6.12

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

The carcinogenic potency of pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide (Aerosil R 972) has been evaluated through a 2-year oral carcinogenicity study in rats.

Only one dose was tested in this carcinogenicity study, 100 mg/kg bw/d and no adverse effect was identified. Although some deficiencies are observed, such as the single tested dose, the low number of tested animals, the lack of statistical test and the lack of control group (comparison with historical controls), this study provides some supportive evidence that these types of material are void of any significant carcinogenic potential by way of ingestion.

No study with a second species was available. Silicon dioxide is a worldwide accepted food additive for animals (US EPA…) and no systemic effect were observed in the available studies. Therefore, the oral route is of no concern. Furthermore, it is not expected that a study in a second species would demonstrate a highest sensitivity.

A published epidemiological study including workers exposed by inhalation to amorphous silicon dioxide is available (Table 17).

The aim of the study was to assess the health impacts of chronic exposure to synthetic amorphous silica (SAS). The study population consisted of 497 subjects exposed to synthetic pyrogenic or precipitated amorphous silica from five SAS producing plants in Germany and 206 non-exposed volunteers selected from white collar workers, health care, fire fighters, technicians, laboratory workers, plant security officers and others.

The prevalence of chronic bronchitis was within expected ranges but slightly higher in exposed subjects (8.7 % in controls vs 11.7% in exposed groups). Tests of pulmonary function showed that air flow values, median FVC (forced vital capacity) and FEV_1 (forced expiratory volume in one second), were somewhat lower in workers exposed to silica (except for plant 4) but there was no difference in the FEV_1/FVC ratio. Additionally, chest radiography showed no increased risk of pneumoconiosis of exposed subjects. In conclusion, this health survey gave no evidence of long-term pulmonary effects after exposure to synthetic amorphous silica.
Nevertheless, the specificity of the substance (surface-treated or not) was not known, no information has been provided regarding the characterisation of particles (particle size) and no data was available concerning the duration and the level of exposure of the workers. Therefore, due to this lack of data, this survey study should be considered with cautious and only in an informative way.

According to the IARC, amorphous silica is not classifiable as to its carcinogenicity in humans (Group 3)[1].

Moreove, the mutagenicity studies suggest negative results, despite some deviations from guidelines.

[1] IARC Group 3 carcinogen: *not classifiable as to its carcinogenicity to humans*. « This category is used most commonly for agents for which the evidence of carcinogenicity is *inadequate* in humans and *inadequate* or *limited* in experimental animals. Exceptionally, agents for which the evidence of carcinogenicity is *inadequate* in humans but *sufficient* in experimental animals may be placed in this category when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans. Agents that do not fall into any other group are also placed in this category. An evaluation in Group 3 is not a determination of non-carcinogenicity or overall safety. It often means that further research is needed, especially when exposures are widespread or the cancer data are consistent with differing interpretations » *(from http://monographs.iarc.fr/ENG/Preamble/CurrentPreamble.pdf).*

10.9.2 Comparison with the CLP criteria

No treatment-related development of tumor was observed.

The criteria for classification as carcinogenic under regulation (EC) 1272/2008 are not fulfilled.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Based on the results of the oral carcinogenicity study, no classification is proposed for the active substance.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The carcinogenicity potential of SAS-HMDS was examined in an oral feeding study with SAS-DDS (A6.7). The DS proposed no classification for carcinogenicity based on the negative results of the study, on the lack of evidence for long-term pulmonary effects after exposure to SAS in an epidemiological study (A6.12) and on the IARC review (1997) which concluded that non-surface treated SAS should be classified as a non-carcinogen (Group 3). Moreover, SAS substances do not display mutagenic properties.

Comments received during public consultation

Comment 1

One MSCA noted that no information was provided on the carcinogenicity after inhalation exposure. There is a concern for carcinogenicity after inhalation considering the increase in 8-OH-guanine DNA adducts in the lung. In addition, the provided oral study has several limitations. Therefore, it should be made clear that the conclusion for no classification is based on absence of data.

Comment 2

A second MSCA emphasized that the data do not allow to make a conclusion on the carcinogenic potential of SAS-HMDS.

Assessment and comparison with the classification criteria

The open literature review on carcinogenicity studies with SAS have only been conducted using the hydrophilic forms and as a result RAC will not consider these for the evaluation of SAS-HMDS.

Table: *Carcinogenicity studies in the CLH report*

Type of study / Reference / Year	**Method Test substance**	**Observations**
Oral feed Wistar rats	No guideline. The study was conducted in 1969; at that time,	Males/Females: At the end of the experimental period, the body weight of the treated animals was within the range of

20/sex / 1969 / A6.7	no guideline was available. No GLP. Only one dose of 100 mg/kg bw/d, 7d/wk, 24 months Reliability: 2 (CAR) There were major deficiencies in the study since there was only one dose used (low), only 20 animals per sex and no statistical test According to the CAR, the control group consisted of 450 untreated animals from previous studies, which received the same feed as the animals of this study SAS-DDS (Aerosil R972)	normal untreated male rats of previous studies and was therefore inconspicuous. Food consumption was also not affected. Neoplastic findings: No treatment-related development of tumour was observed in the limited investigations conducted. No subcutaneous sarcoma, no pituitary gland tumours and no tumours in testes were seen. A benign mammary tumour (fibro–adenoma) was seen in one male; it was noted in the CAR that such tumour also occur in control Wistar rats . No signs of leukosis were seen. Further details of this study are provided in the text below.

Table: Epidemiological carcinogenicity study in the CLH report

Type of data/report Year Reference	Relevant information about the study (as applicable)	Observations
Health survey (five German plants): 497 exposed workers, 206 not exposed / The cross-sectional study was performed from 1995 – 2000 and relates to the exposure to synthetic amorphous silica without differentiation between hydrophilic and hydrophobic types, but to the most part hydrophilic / A6.12	Concentration unknown Chronically exposed (duration unknown)	This preliminary medical health inspection in five German plants of about 500 workers chronically exposed to amorphous silica revealed no particular adverse health effects on the respiratory tract and lung. The workers had been checked for chronic bronchitis, lung function and for signs of pneumoconiosis by X-ray examination. No tumours. No evidence of long term pulmonary effects.

In the chronic toxicity/carcinogenicity study, the treated animals showed no clinical effects. Three cases of mortality observed on week 21 and 24 of treatment were not considered treatment-related. Body weight measurements showed no statistically significant differences between the treated and untreated animals of previous studies, and food consumption in the treated groups remained unchanged. The haematological parameters showed no treatment-related changes. The slight effect seen in the adrenals was of no toxicological significance.

In necropsy observations, there were signs of chronic bronchopneumonia in 14 cases (7 males and 7 females). No signs of leukosis were seen. The changes reported for the lung and the kidney are known to occur with similar incidences in control animals and were therefore not treatment-related effects. The changes reported for the genital tract of the females (atresic follicles in the ovaries, hyperplasia of the interstitial glandular tissue and slight hyperplasia of the uterine mucosa) also

occurred in control animals and are therefore not treatment-related.

Moreover, 3 males and 6 females showed important fat depots; such depots however were described in the CAR as normal for the rat strain used.

In the oral feed carcinogenicity study there were major deficiencies (A6.7). There were only 20 animals/sex used and only one dose and no statistical test (lack of control group and comparison with historical controls). The dose, 100 mg/kg bw/d, was rather low since in a 6 months oral repeated dose toxicity study (IIA.6.4.1) with SAS-DDS (Aerosil R972), dose of 500 mg/kg bw/d no effects with toxicological significance were observed. According to the guidance for dose selection in repeated dose toxicological studies and carcinogenicity studies the highest dose level should be chosen to identify toxic effects including the principal target organs while avoiding severe toxicity, morbidity, or death of the animals. It is clear that the dose selected for this study, which was conducted prior the development of OECD guidelines, did not fulfil the current requirements (Guidance on the Application of the CLP Criteria, 2017; OECD Draft Guidance Document N° 116).

In the CAR, the study is evaluated as being of reliability 2 (Klimisch). RAC believes that this study has significant methodological deficiencies and used the study only as supporting evidence in a weight of evidence approach.

The epidemiological study (A6.12) has the limitation that the exposure is mainly to hydrophilic SAS which are outside the scope of this evaluation. Furthermore, the concentration exposure and the duration of exposure are unknown, along with any possible use of personal protective equipment. Additionally, it has the general uncertainties associated with epidemiological studies such as the exposure assessment and the limited sensitivity and statistical power to confirm the carcinogenic properties of a substance.

In conclusion, based on the limitations mentioned above and the lack of an inhalation carcinogenicity study although there is a concern since there was an increase in 8-OH-guanine DNA adducts seen in the lung in an *in vivo* genotoxicity and gene mutation assay, RAC does not support the DS' conclusion and **proposes no classification due to insufficient data.**

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 10.10.1.1: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
One-generation Screening within a subchronic	Aerosil R 972	The parental males showed no effects. The parental females showed no effects, and the fertility parameters were	IIIA6.8.2_02

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
feeding study Oral, feed Rat Wistar 10 females; 2 males	0, and 500 mg/kg/d Pre-mating: period: 8 wk before 1st mating and 17 wk before 2nd mating Post-mating period: from gestation to 4 wk post-natal	inconspicuous and within control range. Offspring showed no abnormalities and no differences were seen between treated and untreated groups.	

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

No guideline fertility study was available. A "one-generation reproduction screening" study using Aerosil R 972 revealed no impairment of reproductive performance and foetal development. Furthermore, no adverse effects were observed in reproductive tissues from the sub-chronic studies and the oral chronic/carcinogenicity study.

10.10.3 Comparison with the CLP criteria

No impairment of reproductive performance and foetal development was observed.

Thus, no classification according to regulation (EC) 1272/2008 is required.

10.10.4 Adverse effects on development

Table 10.10.4.1: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
No data, national standards USA Mouse CD1 25	Syloid (Silica gel) Oral, gavage Days 6-15 of gestation	At the highest dose, the skeletal findings observed in fetuses such as incomplete ossifications of sternebrae, of vertebrae of extremities or the sternebrae missing, are not considered as adverse for development. Moreover, dams' mortality occurs at this	IIIA6.8.1_01

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
females/dose	0, 13.4, 62.3, 289 and 1340 mg/kg bw/d	dose. NOAEL for embryotoxic/teratogenic effects = 1340 mg/kg bw/d; NOAEL for maternal toxicity = 289 mg/kg bw/d.	
No data, national standards USA Rat Wistar 25 females/dose (24 in the highest dose treated group)	Syloid (Silica gel) Oral, gavage Days 6-15 of gestation 0, 13.5, 62.7, 292 and 1350 mg/kg bw/d	The treatment of pregnant rats with up to 1350 mg/kg bw test substance from day 6 to day 15 of gestation had no adverse effects on nidation and on maternal or fetal survival when compared to the control group. No effects indicative of teratogenicity were seen. NOAEL for embryotoxic/teratogenic effects = 1350 mg/kg bw/d; NOAEL for maternal toxicity = 1350 mg/kg bw/d.	IIIA6.8.1_02
No data, national standards USA Syrian hamster 23 females/dose (24 in the highest dose treated and the positive control groups)	Syloid (Silica gel) Oral, gavage Days 6-10 of gestation 0, 16.0, 74.3, 345 and 1600 mg/kg bw/d	The treatment of pregnant hamsters with up to 1600 mg/kg bw test substance from day 6 to day 10 of gestation had no adverse effects on nidation and on maternal or fetal survival when compared to the control group. No effects indicative of teratogenicity were seen. NOAEL for embryotoxic/teratogenic effects = 1600 mg/kg bw/d; NOAEL for maternal toxicity = 1600 mg/kg bw/d.	IIIA6.8.1_03
No data, national standards USA Rabbit (Dutch) 11-24 females/dose	Syloid (Silica gel) Oral, gavage Days 6-18 of gestation 0, 16.0, 74.3, 345.0 and 1600 mg/kg bw/d	The treatment of pregnant rabbits with up to 1600 mg/kg bw test substance from day 6 to day 18 of gestation had no adverse effects on nidation and on maternal or fetal survival when compared to the sham control group. No effects indicative of teratogenicity were seen. NOAEL for embryotoxic/teratogenic effects = 1600 mg/kg bw/d; NOAEL for maternal toxicity = 1600 mg/kg wb/d.	IIIA6.8.1_04

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

No study on the effects of pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide on teratogenicity is available.

Nevertheless, teratogenicity studies conducted in four different species (mouse, rat, hamster, and rabbit) with an amorphous non surface-treated silica gel (CAS No. 7631-86-9) could give some supportive information on the teratogenic potential of amorphous hydrophobic silica.

The aim of the surface modification is to block the silanol group in order to reduce the affinity of silica for water. Therefore, it is expected that pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide would not be better absorbed by oral route than non surface-treated silica. Furthermore, the lack of systemic effects of both surface treated silica and non surface-treated silica in oral toxicity studies (based on data submitted in this dossier for the first and on literature for the latter) supports a read-across for systemic toxicity.

No teratogenic effects were observed up to the highest doses (between 1340 and 1600 mg/kg bw/d, according to the species).

At the highest dose, skeletal findings were observed in mice fetuses such as incomplete ossifications of sternebrae, of vertebrae of extremities or the missing sternebrae (no statistical test in the report). As delays of ossification are fully reversible, these observations are not considered to be of adverse nature for the development.

Furthermore, based on the results, the FDA concluded that "*the number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated control*". This conclusion is also present in the ECETOC report[2] and in the SIDS[3] relative to amorphous silica and silicate.

Finally, given the fact that these effects were not found in rabbits, rats and hamsters, that the expected oral absorption is low and given the inherent physico-chemical properties of amorphous silica, there is no indication of a potential for reproductive developmental toxicity.

In conclusion, the amorphous non surface-treated silica gel has no teratogenic potential and this result could support the lack of teratogenic effects expected for the pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide.

Moreover, a screening study for reproductive effects (1-generation study) of Aerosil R 972 has been conducted (Doc IIIA 3.8.2), where no malformations were observed in rat pups at the only tested dose of 500 mg/kg bw/d.

10.10.6 Comparison with the CLP criteria

No teratogenic effects were observed up to the highest tested doses (between 1340 and 1600 mg/kg bw/d, according to the species).

Thus, no classification according to regulation (EC) 1272/2008 is required.

10.10.7 Adverse effects on or via lactation

No study available.

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[2] Synthetic Amorphous Silica (CAS No. 7631-86-9), JACC No. 51, ECETOC, 2006.

[3] OECD SIDS for SIAM 19, concerning Synthetic amorphous silica and silicates, 2004

10.10.8 Conclusion on classification and labelling for reproductive toxicity

Based on the results of the reproductive toxicity studies, no classification is proposed for the pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Adverse effects on sexual function and fertility

The DS noted that no guideline fertility study was available in the CLH report. A "one-generation reproduction screening" study using SAS-DDS (Aerosil R972) revealed no impairment of reproductive performance and foetal development. Furthermore, no adverse effects were observed in reproductive tissues from the subchronic studies and the oral chronic/carcinogenicity study. Based on the above the DS proposed no classification for adverse effects on sexual function and fertility.

Adverse effects on development

The DS stated that although there was no study available for the effects of pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide on teratogenicity, there were four studies in the CLH report conducted in four different species (mouse, rat, hamster and rabbit) with a hydrophilic form of silica (syloid, silica gel, no surface treatment and family CAS No 7631-86-9; sub-class CAS-No 112945-52-5). The DS concluded that although there were foetal abnormalities in skeletal tissues observed in the mouse study, these occurred at the highest dose at which maternal toxicity was also observed.

Moreover, a screening study for reproductive effects (1-generation study) of SAS-DDS (Aerosil R972) has been conducted, where no malformations were observed in rat pups at the only tested dose of 500 mg/kg bw/d.

In conclusion, based on the negative results of both the screening 1-generation study with SAS-DDS and the teratogenicity studies with the hydrophilic, non-surface treated SAS, the DS proposed no classification for developmental toxicity for the pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide SAS-HMDS.

Comments received during public consultation

Comment 1

One MSCA stated that an increase in missing sternebrae was reported for the developmental study in mice at the highest dose but was considered not adverse for development. In the MSCA's opinion missing sternebrae should be considered adverse and would warrant classification. Maternal mortality was also reported at this dose level, therefore, it could be argued that the developmental effect is secondary to the maternal toxicity. However, this requires additional information on the maternal toxicity, such as the number of dead mice and a justification. The

MSCA also raised a concern about the read across from the non surface treated, hydrophilic SAS. In addition, the lower bioavailability of surface treated SiO_2 particles should be better explained, as more hydrophobic substances (i.e. surface treated SiO_2) usually tend to display a higher level of bioaccumulation.

Comment 2

A second MSCA commented both on *sexual function and fertility* and on *the development of the offspring*. More specifically, they noted that there is only one poorly described one-generation screening reproductive toxicity study available with SAS-DDS (Aerosil R972) in the CLH report. Since there were severe limitations of this study (e.g. no test guideline, no GLP, few parameters investigated, only one dose, only 2 males, mating ratio 1:5, mating period 14 days) the negative results are considered to be of limited value and hence not sufficient for concluding on the potential of SAS-HMDS to cause adverse effects on sexual function and fertility.

Regarding the adverse effects on the development of the offspring, the MSCA pointed out that since there is no information on the characterisation of the (hydrophilic) tested material amorphous non surface treated silica (Syloid, silica gel) it is difficult to judge the relevance of the four developmental toxicity studies included in the CLH proposal for the (hydrophobic) surface treated amorphous silicon dioxide (SAS-HMDS).

Moreover, since only examination of external gross abnormalities and no histopathology were conducted on the pups in the screening one-generation reproduction toxicity study, the DS conclusion that there were no malformations in rat pups in this study could not be supported.

Overall, the commenting MSCA considered the available data insufficient to conclude on the potential of SAS-HMDS to cause developmental toxicity.

Additional key elements

There are no guideline studies in the open literature with either the substance under evaluation (SAS-HMDS) or the two accepted read across polymorphs of hydrophobic silica, SAS-DDS and SAS-PDMS. However, there are two studies in the review by Becker *et al*. (2013) regarding reproductive and developmental toxicity. In the first study, silica dimethyl silylate (SAS-DDS, 0, 497, and 509 mg/kg bw/d) was administered to Wistar rats (n = 40/sex) in feed for 6 months, after which the rats were mated (1 male to 5 females). The adult rats were killed and necropsied, and the offspring were observed for external appearance and development. No abnormalities were observed in either generation. The NOAEL was 497 mg/kg bw/d for parental generation.

In the second study (study IIA6.7 referred to in the CLH report for carcinogenicity), SAS-DDS (Silica dimethyl silylate) (100 mg/kg bw/d) was administered to Wistar rats (n = 20/sex) in feed for 24 months, after which the rats were mated (1 male to 5 females). The offspring were adjusted to 5/sex in each litter and allowed to mature. After 7 months, they were mated, and their litters were also adjusted to 5/sex. Both sets of offspring were killed and necropsied. There were no reproductive and developmental toxicity effects observed (Becker *et al*., 2013).

Thus, in two non-guideline studies with SAS-DDS no reproductive and developmental toxicity effects were observed.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

There are no guideline studies in the CLH report with either the substance under evaluation (SAS-HMDS) or the two accepted read across polymorphs of hydrophobic silica, SAS-DDS and SAS-PDMS. However, there is a one generation screening study within a subchronic feeding study with SAS-DDS (Aerosil R972). The results are shown in the Table below.

Study A6.8.2_1

Table: *One generation screening study within a subchronic feeding study*

Type of study / Reference / Year	Method	Observations
One-generation screening study within a subchronic feeding study / A6.8.2_1 / 1965	No guideline, No GLP Substance: SAS-DDS (Aerosil R972) Animal: Wistar rat Number of animals per group: See table below Doses: 0 and 500 mg/kg bw/d Oral feed Duration of exposure before mating: 8 wk before 1st mating and 17 wk before 2nd mating Post-mating period: from gestation to 4 wk post-natal Duration of exposure in general P, F1, F2 males, females: 6 months	Parent males and females Clinical effects: The treated animals were inconspicuous and showed no clinical effects. Body weight: No statistically significant differences between the treated and the corresponding control group were seen. Food consumption: Food consumption in the treated group was similar to that in control. Reproduction performance: See Table below on pregnancy and litter data Peri-postnatal development/lactation: Rearing rates were similar for groups IIa and IIb. Offspring: See Table below on offspring observations.

Table: *Number of animals per group*

Test Group	Sex	Dosing (oral mg/kg bw/d)	Number of animals	Mean initial weight (g)
I	Males	500	20	120 ± 2
II	Females	500	20	124 ± 4
III	Males	No treatment	20	122 ± 2
IV	Females	No treatment	20	126 ± 3
IIa	Females	500	10 (for the reproduction toxicity/teratogenicity study)	120 ± 4
IVa	Females	No treatment	10 (for the reproduction toxicity/teratogenicity study)	124 ± 1

Table: *Pregnancy and litter data*

Test group	**IIa (Treated females)**		**IIa (Untreated females)**	
	First litter	**Second litter**	**First litter**	**Second litter**
Pre-treatment with Aerosil R972 (500 mg/kg bw/day; oral)	8 weeks	17 weeks	-	-
Number of females	10	10	10	10
Number of pregnant females (which have delivered)	9	7	6	7
Number of newborns	91	70	62	60
Mean litter size	10.1 ± 2.0	10.0 ± 2.8	10.3 ± 1.9	8.6 ± 4.2
Mean weight of the newborns (g)	5.6 ± 0.4	5.5 ± 0.7	5.1 ± 0.4	5.3 ± 0.5

Table: *Offspring Observations*

Test Group	**IIa (Treated females)**		**IVa (Untreated Females)**	
	First Litter	**Second Litter**	**First Litter**	**Second Litter**
Pre-treatment with Aerosil R972 (500 mg/kg bw/day; oral)	8 weeks	17 weeks	-	-
Stillborns	0	2	2	1
Runts	0	0	0	0
Abnormalities/Lesions	0	0	3*	0

**In 3 cases (same female), the head showed haematoma*

The study investigated the subchronic oral toxicity of SAS-DDS (Aerosil R972) to rats of both sexes treated over a period of 6 months. Within this study, two groups of 10 females each, IIa (treated) and IVa (untreated) were used for screening of reproduction toxicity and teratogenicity.

In the groups IIa and IVa, one treated male of group I was mated with 5 treated females of group IIa. One untreated male (group III) was mated with 5 untreated females of group IVa. Mating was repeated twice: the first mating was performed after 8 weeks of treatment and was followed by a second mating (same animals) after 17 weeks of treatment. The mating period was 14 days, too long a period, thus not providing reliable data on male mating performance.

The following reproduction parameters for the females were considered: pregnancy, litter size, litter weight, rearing-rate during lactation. Offspring were examined post-partum and weekly during lactation for lesions indicative of teratogenicity, development and body weight. The pups were sacrificed when they were 4 weeks old, and were subjected to gross pathological examination.

Results: The parental males and females showed no effects. The reproduction parameters were inconspicuous and within control range. Offspring showed no abnormalities, and no differences were seen between treated and untreated groups.

Deficiencies: The study did not fulfil current guideline requirements for reproductive toxicity/teratogenicity assessment as only few key reproduction parameters were considered. Mating performance was inadequate, as 14 days is too long to enable reliable conclusions to be drawn about male mating performance and in addition, only two males were used and the mating ratio was 1:5 instead of 1:2.

Data were reported in a summarised form, without providing individual details. Only one concentration was tested, and the choice of the test concentration was not explained. Data on animals, husbandry, maintenance, material and methods were limited.

In four studies described in the CLH report for developmental toxicity conducted with hydrophilic SAS (A6.8.1_01, A6.8.1_02, A6.8.1_03, A6.8.1_04) in the mouse, rat, hamster and rabbit, respectively, no effects on fertility parameters were observed.

In the CAR there was an additional supporting screening report with a hydrophilic polymorph of SAS (CAS No 112945-52-5, synthetic amorphous pyrogenic silica), which was not included it in the CLH report. Although RAC decided that data on hydrophilic forms of SAS will not be included as read across in the evaluation of SAS-HMDS, a short summary of this study is presented hereafter in order to be consistent with the rapporteur member state's approach for developmental toxicity.

Study A6.8.2_2

The study investigated the subchronic oral toxicity of hydrophilic SAS (CAS No 112945-52-5) to rats of both sexes treated over a period of 6 months. Within this study, two groups of 5 females each, were used for screening of reproduction toxicity and teratogenicity.

One treated male was mated with 5 treated females of group and one untreated male was mated with 5 untreated females. Mating was performed after 4.5 months of treatment. The mating period was 14 days, too long to provide reliable data on male mating performance.

Results: The parental males and females showed no effects. The reproduction parameters were inconspicuous and within control range. Offspring showed no abnormalities, and no differences were seen between treated and untreated groups.

Deficiencies: The study had the same limitations as the one with the hydrophobic SAS. The study did not fulfil current guideline requirements for reproduction toxicity/teratogenicity assessment as only few fertility parameters were considered; furthermore, mating was inadequate as 14 days is too long. The number of females per test group were only 5 instead of 20 as recommended. The mating ratio was 1:5 (male:females) instead of 1:2.

Data were reported in a summarised form, without providing specific details, or data on each individual animal; no tabular reporting of individual and mean data on fertility and offspring was provided within the report, and the findings were not assessed statistically.

The test substance was not defined in terms of purity. Only one concentration was tested and the choice of the concentration was not explained.

Data on animals, husbandry, maintenance, material and methods were limited.

In conclusion, the key screening study of the CLH report with SAS-DDS had major deficiencies. In addition, studies in the CLH report with the hydrophilic SAS showed no effects on fertility, but also had major deficiencies. In addition, hydrophilic SAS as testing materials are not accepted for read-across for the substance considered for classification in this opinion. There is some evidence from the supporting studies (subchronic studies, the oral chronic/carcinogenicity study and the studies from the Becker *et al.*, 2013) that the hydrophobic polymorphs of silica do not actually induce any

effects on reproduction. However, RAC considers that an appropriate key study is missing and that the available data are of poor quality.

Thus, **RAC proposes no classification for effects on sexual function and fertility due to inadequate and insufficient data**.

Adverse effects on development

There are no studies in the CLH report for the developmental toxicity effects of SAS-HMDS or its read across hydrophobic SAS analogues. However, there are four teratogenicity studies with the substance syloid, a hydrophilic silica gel with CAS number 112926-00-8, which falls under the general category of SAS and the sub-category of SAS produced by the wet method. These studies were done in four different species. Although RAC has concluded that data on hydrophilic forms of SAS would not be used for read across in the evaluation of SAS-HMDS classification, since there are no other data for developmental toxicity in the CLH report, the studies are presented here for reasons of completeness.

A6.8.1_01

Table: Teratogenicity study: hydrophilic amorphous silica (mouse)

Type of study / Reference / Year	Method	Observations
Teratogenicity study / A6.8.1_01 / 1973	No guideline No GLP Substance: Syloid (Silica Aerogel) hydrophilic amorphous silica CAS: 112926-00-8 Animal: Albino CD-1 Mouse Doses: 13.4, 62.3, 289 and 1340 mg/kg bw/d Gavage Duration of exposure: Treatment was conducted from GD6 to GD15; on day 17, the animals were anaesthetised and subjected to Caesarean section. Deficiencies: Only examination of external gross abnormalities and no histopathology were done on the pups The findings were not assessed statistically. The test substance was not defined in terms of purity.	Maternal toxicity: At the highest dose (1340 mg/kg bw/d) the bw gain was reduced by about 20% and the DS and the RMS noted that 14 out 40 dams died. This mortality was considered treatment related because the cause of the mortality was not identified in the study report and at the lower doses, no mortality occurred. Teratogenic / embryotoxic effects: Foetal abnormalities in soft and skeletal tissues were within the range of the controls. Soft tissue abnormalities were reported for two foetuses of the 1340 mg/kg bw/d group and consisted of respectively one case of meningoencephalocele and one case of short tail. However, the incidences of these abnormalities were within the range of spontaneously occurring effects and at a dose where maternal toxicity was observed. Moreover, at the highest dose, there were skeletal findings observed in foetuses such as incomplete ossifications of sternebrae, of vertebrae, of extremities or sternebrae and hyoid missing, which are considered adverse for development in RAC's opinion. However, these effects were observed at maternal toxicity levels since 14/40 dams died and there was 20% decrease in bw gain at this dose. Consequently, the NOAEL for embryotoxic/teratogenic effects is the highest dose (1340 mg/kg bw/d) and the NOAEL for maternal toxicity is 289 mg/kg bw/d.

In this study, there were two issues of ambiguity and concern. Firstly, whether there is maternal toxicity at the highest dose of the study (1340 mg/kg bw/d) and secondly whether the effects observed are severe enough to warrant classification.

The study was reviewed by ECETOC (2006) and OECD SIDS (2004) and it was concluded that no compound related maternal deaths or significant variations of maternal body weight gain were observed to indicate maternal toxicity. The same opinion was shared by the CAR applicant, while the rapporteur member state of the biocide dossier and the DS of the CLH report interpreted the data differently and noted that there were 14/40 maternal deaths. The study report is not clear, but RAC, based on the data in the Table below on fate summary agrees with the ECETOC interpretation that no deaths occurred during the study. In the Table below the "fate summary" is reproduced from the CAR.

Table: *Fate summary*

Group	Material	Dose ** mg/kg	Total		Surviving at Term	
			Mated	Pregnant	Total	Pregnant[1]
231	Sham	0.0	24	22	24	22
232	Aspirin*	150.0	25	20	24	19
237	FDA 71-48	13.4	27	21	27	21
238	FDA 71-48	62.3	24	22	24	22
239	FDA 71-48	289.0	25	22	25	22
240	FDA 71-48	1340.0	40	26	40	26

The DS does not refer to body weight gain. RAC disagrees with the ECETOC and SIDS reviews. In the Table below the body weight results are shown. At the highest dose there is a 20% decrease in corrected body weight gain (calculations made by RAC, average litter weight for controls 0.9 g, average number of foetuses per dam 11.6; average litter weight for high dose group 0.8 g, average number of foetuses per dam 10.4; data from Table A6.8.1-3 of CAR), which could indicate maternal toxicity levels.

Table: *Maternal body weights*

Average Body Weights*

Group	Material	Dose Level	Day 0	6	11	15	17**
		mg/kg	g				
231	Sham	0.0	29.4	32.3	35.9	44.0	50.1 (22)
232	Aspirin***	150.0	29.2	31.7	34.0	39.0	45.3 (19)
237	FDA 71-48	13.4	28.6	32.3	36.8	43.4	49.1 (21)
238	FDA 71-48	62.3	30.2	32.9	36.8	44.0	50.7 (22)
239	FDA 71-48	289.0	29.8	32.4	36.1	43.1	50.0 (22)
240	FDA 71-48	1340.0	27.0	31.4	34.0	37.2	43.6 (26)

In the study, there were skeletal findings observed in foetuses, such as incomplete ossifications of sternebrae and vertebrae, missing sternebrae and hyoid, observed at either the top or the top two doses. There is no statistical analysis in the study, but the above effects are considered adverse and the incidences were statistically significantly increased compared to the controls.

A6.8.1_02

Table: Teratogenicity study: hydrophilic amorphous silica (rat)

Type of study / Reference / Year	**Method**	**Observations**
Teratogenicity study / A6.8.1_02 / 1973	No guideline No GLP Substance: Syloid (Silica Aerogel) CAS: 112926-00-8 Animal: Wistar rat Doses: 13.5, 62.7, 292 and 1350 mg/kg bw/d Gavage Duration of exposure: Treatment was conducted from GD6 to GD15; on day 20, the animals were anesthetised and subjected to Caesarean section. Deficiencies: Only examination of external gross abnormalities and no histopathology were performed. The findings were not assessed statistically. The test substance was not defined in terms of purity.	Maternal toxicity: There was no maternal toxicity observed since at the highest dose (1350 mg/kg bw/d) there were no deaths and no significant reduction in the bw gain (6%). Teratogenic / embryotoxic effects: The foetal abnormalities in skeletal tissues observed were missing sternebrae and wavy ribs but were similar to control group. NOAEL: 1350 mg/kg bw/d

A6.8.1_03

Table: Teratogenicity study: hydrophilic amorphous silica (hamster)

Type of study / Reference / Year	Method	Observations
Teratogenicity study / A6.8.1_03 / 1973	No guideline No GLP Substance: Syloid (Silica Aerogel) CAS: 112926-00-8 Animal: Syrian hamsters Doses: 16.0, 74.3, 345 and 1600 mg/kg bw/d Gavage Duration of exposure: Treatment was conducted from GD6 to GD10; on day 14, the animals were anesthetised and subjected to Caesarean section. Deficiencies: Only examination of external gross abnormalities and no histopathology were performed. The findings were not assessed statistically. The test substance was not defined in terms of purity.	Maternal toxicity: Body weight data: inconspicuous Fate summary: inconspicuous Teratogenic / embryotoxic effects: The only foetal abnormality observed was the extra sternebrae but this was within control group incidences. The treatment of pregnant hamsters with up to 1600 mg/kg bw/d test substance from GD6 to GD10 had no adverse effects on nidation and on maternal or foetal survival when compared to the control group. No effects indicative of teratogenicity were seen.

A6.8.1_04

Table: Teratogenicity study: hydrophilic amorphous silica (rabbit)

Type of study / Reference / Year	Method	Observations
Teratogenicity study / A6.8.1_04 / 1973	No guideline No GLP Substance: Syloid (Silica Aerogel) CAS: 112926-00-8 Animal: Dutch-belted rabbit Doses: 16.0, 74.3, 345 and 1600 mg/kg bw/day Gavage Duration of exposure: Treatment was conducted from day 6 to day 18 of gestation; on day 29, the animals were anesthetised and subjected to Caesarean section.	Maternal toxicity: Body weight data: inconspicuous Fate summary: inconspicuous Teratogenic / embryotoxic effects: Foetal abnormalities in skeletal tissues were within the range of sham-treated controls. Soft tissue abnormalities occurred with increased incidence in the positive control group (total of 28 cases reported). In the treated group with 345 mg/kg bw/d, one pup displayed meningoencephalocele, anopia, medial rotation of the hind limbs and umbilical hernia. In addition, in the high dose group (1600 mg/kg bw/d), one pup displayed club foot. The incidences of soft tissues

	Deficiencies: Only examination of external gross abnormalities and no histopathology were performed. The findings were not assessed statistically. The test substance was not defined in terms of purity.	abnormalities observed were within the range of spontaneously occurring effects. The treatment of pregnant rabbits with up to 1600 mg/kg bw/d from GD6 to GD18 had no adverse effects on nidation and on maternal or foetal survival when compared to the control group. No effects indicative of teratogenicity were seen.

To summarise the developmental toxicity results of the hydrophilic SAS included in the CLH report, in the mouse study there were effects on the development of the foetuses, such as incomplete ossification of sternebrae and missing hyoid, which were observed even at lower doses and in a dose-dependent manner (Table below). On the other hand, at the high dose only, there were signs of maternal toxicity, but the maternal toxicity was not severe, since there were no deaths associated with the treatment and the reduction in the body weight gain was around 20%. RAC believes that the effects seen were adverse and could not be entirely attributed to maternal toxicity, however the study had major deficiencies and the testing material has not been accepted for read across in the current opinion.

***Table**: Developmental effects in the teratogenicity study with hydrophilic amorphous silica (mouse, A6.8.1_01)*

A6.8.1_01: Teratogenicity study: hydrophilic amorphous silica (mouse)						
Dose (mg/kg bw/d)	Control	Aspirin[1]	13.4	62.3	289.0	1340.0
Incomplete ossification sternebrae	47/19[2]	98/19	37/13	71/18	76/16	82/21
Missing sternebrae	10/6	35/15	10/3	34/11	21/10	56/17
Missing hyoid	10/8	47/15	17/7	42/11	53/15	70/20

[1] Positive control 150 mg/kg bw/d of aspirin
[2] All fractions in the table: Number of foetuses affected/Number of litters affected

In the other teratogenicity studies on rat, hamster and rabbit with the hydrophilic SAS, the number of external, visceral or skeletal abnormalities in the test groups did not differ from controls. There were no compound-related maternal deaths or significant variations of maternal body weight gain observed. Thus, of the four species studied for teratogenic effects with hydrophilic SAS, only in the mouse there were effects seen, but these were mostly at the high dose where there was concurrent maternal toxicity (not adverse).

In conclusion, there was a lack of data for developmental toxicity on the hydrophobic SAS both in the CLH report and in the open literature. Although the read across from hydrophilic SAS to the hydrophobic SAS polymorphs is not accepted in the present opinion, RAC presented and discussed the studies from the CLH report and the CAR on hydrophilic SAS, in order to have a more complete picture for the specific endpoint. The data presented are equivocal but give an indication that the hydrophilic SAS does not possess teratogenicity properties. The effects were only observed in the mouse out of the four species tested, under maternal toxicity conditions (not adverse) and the studies had several deficiencies.

Based on all of the above, RAC proposes **no classification for developmental effects due to lack of data**.

Adverse effects on or via lactation

The DS stated that there were no studies available for adverse effects on or via lactation.

In the one generation reproduction screening study, female rats were administered 500 mg/kg bw/d and the following fertility parameters for the females were considered: pregnancy, litter size, litter weight, rearing-rate during lactation. Offspring were examined post-partum and weekly during lactation for lesions indicative of teratogenicity, development and body weight. The parental females showed no effects, and the fertility parameters were inconspicuous and within control range. Offspring showed no abnormalities and no differences were seen between treated and untreated groups.

There were no clinical signs of toxicity, no mortalities, and no treatment-related findings at necropsy, in short there was no evidence to suggest biologically significant maternal toxicity. There was no indication of impaired nursing behaviour or decreased pup viability during lactation and no effect on pup growth to weaning. The results of the study do not indicate any direct, adverse effect on the offspring due to transfer of the active substance via the milk or to the quality of the milk, although the studies were not specific for lactation effects and the parameters monitored are generic. In addition, the one generation reproduction screening study had deficiencies and no toxicokinetic parameters proving that the substance can be present at potentially toxic levels in breast milk are available. Therefore, RAC proposes **no classification for adverse effects on or via lactation due to lack of data**.

Supplemental information - In depth analyses by RAC

In the CAR there is an additional supporting screening report with a hydrophilic polymorph of SAS (CAS No 112945-52-5, synthetic amorphous pyrogenic silica), which the DS did not include in the CLH report. The data thereof are presented in the Table below:

***Table**: One generation screening study within a subchronic feeding study*

Type of study/ Reference / Year	**Method**	**Observations**
One-generation screening study within a subchronic feeding study / A6.8.2_2 / 1962	No guideline, No GLP Substance: Synthetic amorphous silica, pyrogenic, crystalline-free, hydrophilic SAS with CAS No 112945-52-5 Animal: Wistar rat Number of animals per group: See table 20 Dose: 0 and 500 mg/kg bw/d Oral feed Duration of exposure before mating: 4.5 months before 1st mating and 17 wk before 2nd mating	Parent males and females Clinical effects: The treated animals were inconspicuous and showed no clinical effects. Body weight: No statistically significant differences between the treated and the corresponding control group were seen. Food consumption: Food consumption in the treated group was similar to that in control. Reproduction performance: See Table 21 Peri-postnatal development/lactation: Rearing rates were similar for groups IIa and IIb. Offspring: See table Offspring Observations, under "adverse effects on fertility"

	Post-mating period: from gestation to 4 wk post-natal Duration of exposure in general P, F1, F2 males, females: 6 months	

10.11 Specific target organ toxicity-single exposure

No study available.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The DS stated that there is no study available for this hazard class and thus it was not evaluated in the CLH report. Nevertheless, comments were received during the public consultation, and there were in fact data included in the CLH dossier under other hazard classes (i.e. acute toxicity).

Comments received during public consultation

An MSCA stated that data on single exposure are available from the acute toxicity studies after oral and inhalation exposure, thus they suggested comparing the effects observed in these studies with the STOT SE criteria.

Additional key elements

RAC decided to use data from the acute oral and inhalation studies that refer to surviving animals to assess the substance for STOT SE classification. RAC's approach to the reliability assessment for the open literature studies, as explained under "additional key elements" in the "Acute Inhalation Toxicity" section of this background document, is equally valid for STOT SE. The data relevant for STOT SE is summarised in the following Table:

***Table**: Data from acute oral and inhalation toxicity studies regarding surviving animals with all three forms of hydrophobic SAS available in the open literature and in the CLH dossier*

A/A	Species / Reference/ Year of the study*	Method, Test substance	Clinical and histopathological observations
Oral studies			
1	Wistar Rat /	*OECD TG 401, GLP*	No mortalities observed

	A6.1.1/ Degussa 1981	5/sex/concentration One dose: 2000 mg/kg bw SAS-HMDS (Aerosil R812)	Clinical results: During exposure, the animals were somewhat restless and their eyes were half-closed[2]. Slight dyspnoea after 1 hour exposure[1]. Body weight decreased during the first 2 days of observations, but thereafter body weight gain turned back to normal. Necropsy: Pathology revealed no abnormalities.
Inhalation studies			
1	BR Rat / ECETOC, 2006; Becker *et al*., 2013 / Cabot 1982	*Guideline compliant study with acceptable restrictions* 5/sex Single dose: 2280 mg/m^3 Exposure: 1h Particle size/MMAD*: 0.15 µm SAS-DDS (Cabot) Control group	**2280 mg/m^3** No mortalities observed Clinical signs (during and after exposure): Irregular breathing[1] After treatment: poor coat quality and alopecia in females
2	Wistar rats / ECETOC, 2006; Becker *et al*. 2013 / Cabot 1994a	*Comparable to guideline study* 5/sex Dosing: 210, 540, 2100 mg/m^3 Exposure: 4h Particle size/MMAD: 0.8-1 µm/ 1.175-1.275 µm Surface Area: 130 m^2/g SAS-DDS (Cab-O-Sil TS610)	**210 mg/m^3** No mortalities observed During exposure: closed eyes[2], laboured breathing[1], licking inside of mouth and laying on back After exposure: sporadic instances of few faeces, anorexia, chromodacryorrhea[2], laboured breathing[1], wetness of the nose/mouth area[3], diarrhoea and transient decreases in body weight gain Necropsy findings: darker lungs than normal[4], white and red areas in lungs[4]. **540 mg/m^3** All signs reported during exposure and after exposure (7/10 animals died during exposure, no data on whether the signs observed refer to surviving animals were reversed from days 4 to 14. Body weights in the surviving females decreased on day 7 but had recovered by day 14. Necropsy findings (survivors) Lungs darker than normal with red and white areas[4].
3	BR rats / Becker, 2013; EPA, 2011 /	5/sex, high dose 7/sex Dose: 520, 1120, 2790 mg/m^3	**520 mg/m^3** No mortalities observed During exposure: decreased, irregular

	Cabot 2003 (revised)	Exposure: 4h Particle size/MMAD: 1.24 µm SAS-DDS	breathing[1] After exposure: increased breathing rates[1], laboured breathing[1] and blepharospasm[2], all of which resolved in four days. Necropsy findings: Lungs filled with foam[4]
4	SD rats / ECETOC, 2006 / Wacker 1996	*GLP and guideline compliant study (OECD, EC, EPA, FDA, etc.)* 5/sex Dose: 900, 2200 mg/m^3 Exposure: 4h nose only Particle size/MMAD: 7.2-7.7 µm Surface area: 130 m^2/g SAS-HMDS HDH SKS130	Note Same test as above with the same SAS with higher MMAD was carried out. **900 mg/m^3** No mortalities observed 1/5 male and 2/5 females showed trace red discoloration of the lungs[4] **2200 mg/m^3** All animals that survived (6/10) were within normal limits
5	Wistar Rat / A6.1.3 / Degussa 1983	*GLP, No guideline method* *Reliability 2 (Klimisch)* 5/sex/concentration One dose: 477 mg/m^3 The particle size distribution of the inhalable fraction revealed that about 56 % of the particles had an aerodynamic diameter <5 µm (respirable). MMAD = 2.9 µm Whole body, 4 hour exposure SAS-DDS (Aerosil R974)	**477 mg/m^3** No mortality observed Clinical results: During exposure, the animals were somewhat restless and their eyes were half-closed[2]. Body weight decreased during the first 2 days of observations, but thereafter body weight gain turned back to normal. Necropsy: Pathology revealed no abnormalities.

* *The references are to the review articles where the studies are mentioned, as well as the source and year of the study*

** *Becker et al. (2013) provides particle size dimensions in µm; ECETOC (2006) provides particle size MMAD (calculated by Cascade impactor) in µm; MMAD is defined as the aerodynamic diameter at which 50% of the particles by mass are larger and 50% are smaller*

[1] *Clinical signs of various pathologies possibly associated with respiratory tract abnormalities*

[2] *Clinical signs associated with the peripheral/ autonomous nervous system*

[3] *Clinical signs associated with upper respiratory tract irritation*

[4] *Findings associated with respiratory tract abnormalities*

In addition, from the mechanistic study A6.10 (2005) using Wistar rats described in the CLH report, symptoms indicative of inflammation in the deeper areas of the lung were reported at the start of the observation period, but were fully reversible within the end of the experiment. Neither fibrogenic nor tumorigenic effects or chronic processes were observed at the concentrations tested.

Assessment and comparison with the classification criteria

Some slight clinical effects indicating generalised stress caused by an unwell condition (ruffled fur, poor coat quality and alopecia in females) at high doses (oral 2000 mg/kg bw and 2280 mg/m^3 inhalation, well above the LC_{50}) are not specific for any particular pathology and could be secondary effects, as discussed below. Other clinical symptoms mainly correlated with nervous system abnormalities, such as chromodacryorrhea and blepharospasm, are observed in one study and one dose (210 mg/m^3, which is 1/2 of the LC_{50}), and although definitely linked to exposure, they were not sufficiently adverse to support classification for STOT SE.

Clinical signs, which included slight sedation or restlessness, hunched position or laying back, eyes half-closed and anorexia were observed both in oral and inhalation studies, but were considered weak and no specific pathology was identified. In addition, such clinical symptoms could have multi-factorial aetiology, such as decreased oxygenation, as discussed later in this section. Therefore, and taking into consideration the chemical structure of silanamine, which does not raise any alerts as a psychoactive compound with sleep-inducing properties, they do not constitute a basis for classification as STOT SE 3 for narcotic effects.

One of the most prominent and consistent clinical sign observed in all acute inhalation studies in surviving animals or in studies where no deaths are reported, was irregular/laboured breathing, at doses starting from 210 mg/m^3 (1/2 of the LC_{50} for inhalation) up to 2280 mg/m^3. Even at 90 mg/m^3 (1/5 of the LC_{50} inhalation) in the acute inhalation study by Cabot (1994), laboured breathing is implied only as a clinical finding from the statement "*Similar results were observed with Cab-O-Sil TS530*", but very few details are provided in the ECETOC (2006) report, where the study is mentioned. Unfortunately, no data on single inhalation exposure are available for lower doses. Slight dyspnoea was also observed at 2000 mg/kg bw after 1 hour oral exposure. Wetness of the nose/mouth area was also reported after inhalation of silanamine. The most common gross necropsy finding was darker lungs and white/red areas (discoloration) in the lungs (at 210 and 900 mg/m^3), indicating congestion and pulmonary haemorrhage, depending on the extent of discoloration (López, 2012). At 540 mg/m^3 lungs were found full of foam probably caused by the presence of particulates in the lung (described by Lewis *et al.,* 2013), indicating pneumonic oedema. Unfortunately, no histopathology data were available. All effects point to lung dysfunction. The clinical signs linked to lung dysfunction appeared during exposure and persisted for a few (four) days after exposure and then they gradually reversed. The mechanism involved it is believed to be local inflammation, as suggested by the findings of the mechanistic study of the CLH dossier (A6.10, 2005) and the histopathology findings in some other studies.

Therefore, RAC considers that although the cluster of symptoms described above are all connected with the respiratory system, and more specifically with lung dysfunction, the doses where effects (clinical symptoms and necropsy findings) were observed in non-dying animals were close to (approximately half of) or above the LC_{50} for acute inhalation, based on the set of data available in this opinion. Consequently, **no classification for STOT SE is warranted for silanamine**.

10.12 Specific target organ toxicity-repeated exposure

Table 10.12.1: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
No guideline study, it was performed in 1964 No GLP Rat, Wistar, 5 males and 5 females	Aerosil R 972 Oral, feed 5 weeks; 8 weeks (high-dose group) 7 d/wk 0, 500, 1 000 and 2 000 mg/kg bw/d) (the 2000 mg/kg bw/d successively increased to 16 000 mg/kg/d)	**Low dose:** no effects observed **Medium dose:** at 1000 mg/kg bw/d and above: liver atrophy (2/10 rats), loss of basophilic structure and diminution of the glycogen content in the hepatocytes. **High dose:** at 2000 mg/kg bw/d: shrunk and hyperchromatic nuclei of the hepatocytes **Higher** : at 16 000 mg/kg/d: loss of bodyweight gain, emaciation, cachexia, mortality	IIIA6.3.1
No guideline study, it was performed in 1964 No GLP Rat, Wistar, 20 males and 20 females	Aerosil R 972 Oral, feed 6 months 7 d/wk 0, 500 mg/kg/d	The treated animals were inconspicuous and showed no clinical effects. No statistically significant differences between the treated and the corresponding control groups were seen regarding mortality and mean body weight. Food consumption in the treated groups was similar to that in controls. The haematological parameters showed no treatment-related changes. No significant differences in organ weights between treated and corresponding control groups were seen. Neither gross- nor histopathological examination revealed treatment-related abnormalities. However, histopathology revealed a slight progressive change indicative of a chronic stress-reaction in the adrenals of treated animals. In females subjected to a post exposure period of 3 weeks, the effect seen in the adrenals turned back to normal, indicating reversibility. These effects were considered of no toxicological significance.	IIIA6.4.1

No guideline study, it was performed in 1969 No GLP Rat, Wistar, 20 males and 20 females	Aerosil R 972 Oral, feed 24 months 7 d/wk 0, 100 mg/kg/d	No mortality was observed. None of the treated animals showed clinical signs of toxicity. There were signs of chronic bronchopneumonia in 14 cases (7 males and 7 females). No signs of leukosis were seen. The changes reported for the lung and the kidney are known to occur with similar incidences in control animals and were therefore not treatment-related effects. The changes reported for the genital tract of the females (atresic follicles in the ovaries, hyperplasia of the interstitial glandular tissue and slight hyperplasia of the uterine mucosa) also occur in control animals and are therefore not treatment-related. Moreover, 3 males and 6 females showed important fat deposit; such deposit however are considered to be normal for the rat strain used. No treatment-related development of tumor could be observed.	IIIA6.5
No guideline study No GLP Rat, Wistar, 10 males and 10 females	Aerosil R 974 Inhalation 14 d (preliminary test) 6h/d 5 d/wk 0, 31, 87, and 209 mg/m^3 (analytical)	**Low dose:** Respiratory distress; Histological changes in lungs related to alveolar inflammatory response (bronchiolar mucous cell proliferation and increased cellularity, accumulation of alveolar macrophage, alveolar oedema and early granuloma); Increased lung weight **Medium dose:** Slight to moderate dyspnea; Haematological effects (increased red blood cell count, haemoglobin content and packed cell volume). **High dose:** Mortality 6/20 (4 m + 2 f)	IIIA6.3.3

No guideline mentioned, but comparable to guideline study (acc. to OECD Guide-line 413) GLP Rat Wistar 70 males and 70 females, sub-groups of 10 males and 10 females	Aerosil R 974 Inhalation 13 wks (recovery period: up to 52 wks) 6h/d 5 d/wk 0, 35 mg/m^3 (analytical), (total dust)	Increased lung weight; Inflammatory signs such as nasal irritation; Granuloma like lesions; Accumulation of alveolar macrophages; Leukocytosis; Signs of interstitial fibrosis with increase of the lung collagen content. Si deposit in lungs and in lymphatic mediastinal nodes. No mortality. No particular clinical signs. **Recovery**: septal cellularity still present at the end of the recovery period. The other changes appear reversible.	IIIA6.4.3_01

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Repeated dose toxicity studies were available for Aerosil R 972 *via* oral route and for Aerosil R 974 *via* inhalation (Please refer to Doc IIIA for details).

Oral administration:

No concern arose from a sub-acute oral exposure.

Rats were exposed to 500, 1000 mg/kg/d of Aerosil R 972 for 5 weeks (Doc IIIA6.3.1). Another dose group was tested using an escalating method with a starting dose of 2000 mg/kg bw/d for 2 weeks. Because of good tolerance of the 2000 mg/kg bw dosing, this dose was increased after 2 weeks to 4000 mg/kg bw/d, after further 2 weeks to 8000 mg/kg bw/d and finally the dose was increased to 16000 mg/kg bw/d for 2 weeks. Therefore, the exposure period for this dose group was extended to 8 weeks. The experimental period of the control group also was extended to 8 weeks.

A loss of body weight gain was noted at 8000 mg/kg bw/d and above.

At 16000 mg/kg bw/d, animals died by emaciation and cachexia. The histological analysis showed hepatic effects at 1000 mg/kg bw/d and above (2/10 animals). These effects were characterised by an occasional atrophy of the liver epithelium, a loss of basophilic structure and a diminution of the glycogen content in the hepatocytes. These liver effects were considered to be related to starving and not being systemic effects provoked by the Aerosil R 972, at the dose of 16000 mg/kg bw/d.

At 1000 mg/kg bw/d (corresponding to 1.5 – 2 % in the feed), after 5 weeks, the link between these liver effects and the substance (systemic effect or starving) was not evident. Furthermore, silicon dioxide is a worldwide accepted food additive and no systemic effects were observed in the other submitted oral studies.

Finally, since several deficiencies were noted (no individual data, no control group and no statistical test), the study seemed to be irrelevant to conclude that the tested substance could have a liver systemic toxicity.

In a 6-month feeding study in rats receiving only one dose-level of Aerosil R 972 (500 mg/kg bw/d), there was no treatment-related findings (Doc IIIA6.4.1).

Aerosil R 972 was administered to rats in a 24-month feeding study at the only dose level of 100 mg/kg bw/d (Doc IIIA6.5).
Some effects observed in the lung, the kidney and in the genital tract of the females of treated groups were also observed in the control group and are therefore considered as not treatment-related.
Although several deficiencies such as the low number of tested animals, the absence of statistical test, the only one tested dose and the lack of control group (comparison with historical controls), the study was considered as supportive data for this endpoint.

No study with a second non-rodent species was available.
As already stated, silicon dioxide is a worldwide accepted food additive for animals (US EPA…) and no systemic effect were observed in the available studies. The low systemic toxicity of the pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide can be a result of its limited absorption or it relates real lack of toxicity. It is not expected that studies in a non-rodent species would demonstrate a higher sensitivity.

Dermal administration:

No data is available. However, considering the lack of local effects observed in irritation studies, only systemic effects may be expected.
Nevertheless, since no systemic effects were observed in oral studies (see above) and because of the low potential of dermal penetration, it is considered that no hazards are expected by dermal administration.

Administration by inhalation:

The 90-day inhalation study (doc IIIA6.4.3_1) compared the toxicity of three amorphous silica: Aerosil 200 (fumed silica), Aerosil R 974 (pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide) and Sipernat 22S (precipitated silica) with quartz dust.

Rats were exposed to 1, 6 or 30 $mg_{Aerosil\ 200}/m^3$, 35 $mg_{Aerosil\ R974}/m^3$, 35 $mg_{Sipernat\ 22S}/m^3$ or 60 mg_{quartz}/m^3 in inhalation chambers for 6 h/day, 5 days/week for 13 weeks. Only Aerosil R 974 was considered further for assessing the toxicity of the notified surface-treated silica.

The unique dose of 35 mg/m^3 (analytical value) of Aerosil R 974 was determined in a range finding study run during 14 days (doc IIIA6.3.3).
The study is comparable to guideline study and is GLP: the number of animals (10 animals/sex/ group), the duration of exposure (90 d, 6h/d, 5 d/week), the experimental conditions, the observation of clinical effects, the evaluation of haematological parameters, clinical chemical parameters, urinalysis and the examination at necropsy, all these assessment parameters are similar to the guideline. However, particle size determination in test atmospheres could not be performed due to electrostatic charge of the particles.

Rats were killed for observations after the exposure period and 13, 26, 39 and 52 weeks after exposure. Clinical signs, body weight, haematology, biochemistry, urinalyses, organ weights, retention of test material in the lungs and the regional lymph nodes, collagen content of the lungs and gross and microscopic pathology were determined.

Males exhibited statistically significantly lower body weights in weeks 6 to 9 only. Haematological changes (increased red blood cell counts, haemoglobin contents, packed cell volumes and prothrombin time) were observed in males at the end of the exposure period only. These changes can be probably considered as a compensative hyperaemia, result of the

impaired lung function. The following effects were observed in the lungs of rat exposed to 35 mg/m^3 of Aerosil R 974:

- increased lung weight noted at the end of the exposure period but normal after a recovery period of 3 months;
- swollen and/or spotted lungs and irregular surface of the lung in some animals at the end of exposure and after 13 weeks;
- accumulation of alveolar macrophages in most males and females after observation periods of 13, 26 and 39 weeks but not seen after 52 weeks of recovery;
- intra-alveolar accumulation of granular material, cellular debris and leucocytes infiltration observed at the end of the exposure period but not found anymore after 13 weeks of recovery;
- granuloma-like lesions seen in all animals at the end of the exposure period and after an observation period of 13 weeks. These lesions did not show fibroblastic activity and hyalinization in the granulomas. This lesion decreased in incidence and was not found anymore after an observation period of 52 weeks;
- increase in the lung collagen observed in males and females at the end of the exposure period. Although the differences gradually decreased during the recovery period, they remained statistically significant after observation periods of 13 and 39 weeks;
- some signs of focal interstitial fibrosis observed in 3/5 male rats after 13 weeks of the recovery period and in 1/5 male rat after 26 weeks of recovery period (not statistically significant);
- increased septal cellularity still present in a few animals after an observation period of 52 weeks (very slight degree);
- alveolar bronchiolisation in 2/10 males after the exposure period and in 1/5 at 13 week post-exposure (not statistically significant);
- high amount of silicon detected in the lungs and lymph nodes of males and females at the end of the exposure period and after observation periods of 13 and 26 weeks. Silicon was still present in lymph nodes of one male at the end of the observation period.

Nasal inflammatory signs such as nasal irritation, focal necrosis and rhinitis and slight degeneration of the olfactory epithelium, were also reported.

In conclusion, the lung was the major target organ after exposure to Aerosil R 974.
All the observed effects were characteristic of an inflammation and were reversible. They completely disappeared at the end of the one-year recovery period, except septal cellularity which was still present in 2 animals of each sex.

The effects could be mainly related to a pulmonary overload and no dose-response relationship could be established. Similar phenomenon in the generation of an alveolar inflammation was observed in preliminary 14-day study in rats (III6.3.3). This supports the conclusions that lung is the target organ after exposure to amorphous silica.

No study with a second non-rodent species was available. It is acknowledged that rats have a more protective upper respiratory surface area compared to human, the observed effects in rat lungs lead to consider that human lungs and especially alveolar part, could be more severely exposed to silica. Rat remains the most suitable species to predict lung toxicity.

Finally, in the mechanistic *in vivo* assay (Doc IIIA.6.6.4), Aerosil R 812 S was given by a single intra-tracheal injection to rats and followed by a 90 day post-exposure period. The study focused on observed lung damages and markers of toxicity after exposure of rats to

amorphous Aerosil R 812 S, compared to the positive lung carcinogen, a crystalline silica (quartz) dust.
An increase of the 8-OH-guanine level is observed, it could therefore be assumed that a chronic exposure to high level of Aerosil R 812 S could lead to a saturation of the DNA repair mechanism that could give rise to the occurrence of mutations.
Moreover, the fact that mutant protein p53 was not detected by a specific antibody does not ensure that no mutation was produced in DNA. Nevertheless, it was observed in the study that Aerosil R 812 S induced a response clearly different from that of quartz.

10.12.2 Comparison with the CLP criteria

A classification STOT RE 2 H373 (May cause damage to organs through prolonged or repeated exposure) according to the CLP regulation is proposed for pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide ; based on the slight to moderate significant increase of the lung collagen content with signs of focal interstitial fibrosis, on the granuloma-like lesions and on septal cellularity (still present at 52 weeks of recovery) after inhalation exposure to Aerosil R 974.
Even if the majority of these effects were reversible during the one-year recovery period, it is considered that the time necessary for the reversibility is relatively important compared to the duration of the exposure.

According to the CLP regulation (1272/2008), these findings meet the following criteria for classification STOT RE 2: "*significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g. sight, hearing and sens of smell)* (criteria b) *or multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity* (criteria e).
Classification is based on observed effects and not on potential expected effects. The threshold value is defined by the effects observed at a dose between 20 and 200 mg/m^3 in rats, during 6h/d, which is the case in this study.

Finally, even if there is no long-term respiratory health effect in the available epidemiological study in workers (section 10.9_Table 50), uncertainties are present in this publication (nature of the silica, duration and level of the exposure) leading to inadequate evidence. In this context, the epidemiological study cannot be used as a proof of no effect and cannot rule out the pulmonary effect reported in rats.

10.12.3 Conclusion on classification and labelling for STOT RE

Based on the results of the 90-d inhalation rat study, a classification STOT RE 2 – H373 is proposed for the active substance.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The toxicity of silanamine following repeated exposure has been evaluated by the DS based on three oral and two inhalation studies, all in Wistar rats.

Oral studies

In the subacute study (A6.3.1) with SAS-DDS (Aerosil R972) liver was the target organ in Wistar rats but the DS concluded that due to the significant deficiencies of the study, it could not be concluded whether the tested substance could have liver systemic toxicity. In the subchronic study (A6.4.1) with SAS-DDS (Aerosil R972) the only effect observed was a reversible stress reaction in the adrenals of the treated rats. The effect was considered of no toxicological significance. This study also had significant deficiencies. In the chronic/carcinogenicity study (CAR, carcinogenicity section, A6.5) the target organs were the lungs, the kidney and the genital tract of the females but all these effects were also seen in the control animals and, as a result, the DS concluded that the effects were not treatment related. This study also has significant deficiencies.

In conclusion, the DS stated that since silicon dioxide is a worldwide accepted food additive and no systemic effects were observed in all the submitted oral studies no classification is warranted for repeated dose toxicity based on the oral studies.

Inhalation studies

In the preliminary 14d study (A6.3.3) with SAS-DDS (Aerosil R974) the target organ was clearly the lung, since at all doses respiratory distress, dyspnoea and histological changes to the lung related to alveolar inflammation were observed. In the subchronic, 90d inhalation study (A6.4.3) with SAS-DDS (Aerosil R974), the lung again was the target organ with the following findings:

- Increased lung weight (reversible)
- Swollen and/or spotted lungs (reversible)
- Accumulation of granular material, cellular debris and leucocytes infiltration (reversible)
- Granuloma-like lesions (reversible)
- Increase in lung collagen (reversible)
- Signs of focal interstitial fibrosis (reversible)
- Increased septal cellularity/slight effect (irreversible)
- Alveolar bronchiolisation (reversible)
- High amount of silicon detected in lungs (reversible)

Nasal inflammatory signs such as nasal irritation, focal necrosis and rhinitis and slight degeneration of the olfactory epithelium, were also reported. In conclusion, the DS stated that the lung was the major target organ after exposure to SAS-DDS (Aerosil R974). Nearly all the observed effects were characteristic of inflammation and were reversible. They had completely disappeared at the end of the one-year recovery period, except septal cellularity which was still present in 2 animals of each sex.

Based on the slight to moderate significant increase of the lung collagen content with signs of focal interstitial fibrosis, on the granuloma-like lesions and on septal cellularity (still present at 52 weeks of recovery) after inhalation exposure to SAS-DDS, the DS proposed to classify silanamine as STOT RE 2, H373 (May cause damage to organs through prolonged or repeated exposure,

lungs via inhalation).

Comments received during public consultation

Regarding the evaluation of the STOT RE endpoint, six comments were received:

Two were from industry associations, two from individuals and two from MSCAs.

One industry association noted that the classification is based on effects characteristic of inflammation and were reversible. Additionally according to them, the effect could be mainly related to a pulmonary overload and no dose-response relationship could be established for the study. These effects were not considered to be intrinsic to the substance but common to "poorly soluble low toxicity particles". They argued that there should be no classification of substances in the CLP Regulation based on results of this type.

A second industry association stated that in the CLH report crucial information was not included. More specifically, the re-analysis of the lung tissue slides of the original study by Reuzel *et al*. (1991) conducted by an expert pathology working group was not discussed in the CLH dossier (Weber *et al*., 2018). This re-analysis clearly demonstrated that focal interstitial fibrosis, an irreversible disease, was not present in the lungs of the SAS-DDS (Aerosil R974) exposed rats at any point in time. The study pathologist of the original study agreed with the outcome of the review upon re-evaluation of the original lung slides in a subsequent statement. Therefore, this commenting industry association affirmed that the effects observed with SAS-DDS (Aerosil R974) represent markers of typical inflammatory responses of the rat lung after continued high exposures to particles, which may persist over a long time (ECETOC, 2006), these markers are fully reversible and cannot be termed adverse. Accordingly, the conditions that would trigger a STOT RE 2 classification have not been met. The same industry association noted that the CLH report does not consider the value of existing animal inhalation studies with similar SAS materials or epidemiological studies done in SAS production plants. The issue of SAS clearance from the lung was also raised.

One comment from an individual emphasised the re-analysis of the original key study by Weber *et al.* (2018), which was missing from the CLH report: the re-analysis shows that the changes in the lungs of SAS-DDS (Aerosil R974)-exposed animals were not considered adverse because they are reversible; therefore "serious changes to the biochemistry or haematology of the organism" have not been shown. In addition, the commenting individual stated that a large number of occupational epidemiology studies do not give any indication for adverse lung effects in workers with occupational exposure to SAS. Therefore, a classification of the substance as STOT RE 2, H373 is not warranted and is inconsistent with ECHA guidance and the EU regulation.

The second individual's comment was similar, emphasising the Weber *et al.* (2018) re-analysis study, as well as the epidemiological studies. This commenting individual also stressed that the rapid clearance of the SAS particles shows they are not poorly soluble particles and thus would not cause the physio-pathological phenomenon called "lung overload", which is known to cause persistent lung epithelial cell proliferation. SAS particles do not meet the "low soluble" criterion. Thus, a STOT RE classification is not required.

One MSCA agreed that the results of the study presented in the CLH dossier justify classification in category 2, but classification in category 1 cannot be excluded, because no group is available with exposures below 35 mg/m^3. Information from the 14 day range-finding study showed that lung function was severely affected. Therefore, the effects observed at 80 mg/m^3 warrant

classification as STOT RE 1.

The second MSCA agreed that a classification in STOT RE 2, H373 (lung) for SAS-HMDS is warranted. Moreover, the commenting MSCA pointed out that the results from the available negative epidemiological study cannot be used as evidence of no effect and cannot rule out the pulmonary effect reported in rats.

Additional key elements

Oral studies

In the open literature there is an additional oral study (Degussa, 1962; Becker *et al.,* 2013*)*, where SAS-DDS (500 or 1000 mg/kg bw/d) was orally administered to Wistar rats (n = 40/sex) by gavage for every other day for 19 or 39 days. The rats were killed and necropsied at the end of the treatment period or after 4 weeks of recovery. There were no clinical signs or treatment-related effects. The no-observed adverse effect level (NOAEL) was 1000 mg/kg bw/d.

Inhalation studies

In the open literature there are additional studies (not included in the CLH report) regarding the repeated dose toxicity of hydrophobic surface treated silica. These studies are presented in the Table "Inhalation repeated dose toxicity studies with all three forms of hydrophobic SAS available in the open literature and in the CLH dossier" under "Assessment and comparison with the classification criteria".

Assessment and comparison with the classification criteria

In the following Table, a summary of all relevant repeated dose toxicity studies with hydrophobic SAS from the CLH report as well as the open literature is shown, focusing mainly on the effects on lungs. RAC's approach to the reliability assessment for the open literature studies, explained under "additional key elements" in the "Acute Inhalation Toxicity" Section of the background document, is equally valid for STOT RE.

Table: Inhalation repeated dose toxicity studies with all three forms of hydrophobic SAS available in the open literature and in the CLH dossier

A/A	Species / Reference / Year of the study*	Method/ Test Substance	Results*
Inhalation Studies			
1	Wistar rats (10/sex) / A6.3.3 / Degussa, 1986	No guideline, no GLP study, reliability 1 (Klimisch) SAS-DDS Aerosil R974 Doses (mg/m^3): 0, 31, 87, 209 (nominal concentration 450 mg/m^3, lowered to a measured value of 209 mg/m^3 due	✓ Mortality: 4 males and 2 females of the highest dose group died The males died during the first 24 hours following the first exposure, whereas the two females died on day one after the first exposure, after reduction of the test concentration to 209 mg/m^3 ✓ Body weight: significant decrease at 87 and 209 mg/m^3 (12.6-35.5% and 26.4-42.8% at 7-14 days, respectively) for males with

		to deaths) 14 days, 6h/d, 5d/wk	significant concomitant decrease in food consumption reaching even 75% at 14 days. Effects in females were similar but less pronounced. Clinical signs: In all treated test groups, the animals mainly suffered from respiratory distress. At 87 mg/m^3, the animals showed slight to moderate dyspnoea. In 31 mg/m^3 the animals showed no effects. Necropsy findings (in nearly all animals of all treated groups, at all doses, but more pronounced at 209 mg/m^3) Increased lung weight Lungs: paleness, swelling, spotting and/or spongy surface; occasional small focal haemorrhages Bronchiolar mucous cells proliferation increased cellularity Accumulation of alveolar macrophages, alveolar oedema, early granuloma Focal increased septal cellularity (mainly consisting of macrophages and lymphocytes aggregates) Granulomas (mainly consisting of macrophages and lymphocytes aggregates) Haematological effects (87 and 209 mg/m^3) Increased red blood cell count (5.1% and 11.9%), haemoglobin content (7.5% and 15%) and packed cell volume. LOAEC: 31 mg/m^3 (based on inflammatory responses in the lung) Criteria for classification# – inhalation STOT RE 1 ≤ 120 mg/m^3
2	Wistar rats, (70/ sex) / A6.4.3_01; Reuzel et al., 1991 / Degussa, 1987	Comparable to guideline study OECD TG 413, GLP study, reliability 2 (Klimisch) SAS-DDS Aerosil R974 Doses (mg/m^3): 0, 35 13 wks 6h/d, 5d/wk Recovery period 52 wks No particle size determination performed	Original Observations No mortality. No particular clinical signs (In the Reuzel et al., 1991 study, though, it is stated that "Respiratory distress was observed in all rats exposed to Aerosil R 974") Increased lung weight The lungs were swollen, spotted, and showed a spongy or irregular surface; the lymph nodes were enlarged. However, after a post-exposure period of 26 weeks, these effects disappeared. Inflammatory signs such as nasal irritation; Granuloma like lesions; Accumulation of alveolar macrophages; Leukocytosis; Signs of interstitial fibrosis with increase of

			the lung collagen content. Si deposit in lungs and in lymphatic mediastinal nodes. Histopathology of the nose revealed: Focal necrosis and slight atrophy of the olfactory epithelium after 13 weeks of exposure and 13 weeks post-exposure, but was no longer observed during the remainder of the recovery period. Recovery: septal cellularity still present at the end of the recovery period. The other changes appear reversible. LOAEC: 35 mg/m^3 (measured, highest dose tested) Criteria for classification$^{\#}$ – inhalation STOT RE 2 ≤ 200 mg/m^3
	Weber et al. 2018		<u>Revised histopathological observations</u> **End of exposure, Males $^{¥}$** 10 animals Alveolar macrophages n=10/2.7$^{\&\wedge}$ Macrophage aggregations n=10/1.4$^{\&\wedge}$ Pneumocyte type II hyperplasia n=9/1.9$^{\&\wedge}$ Granulomatous inflammation n=10/3.5$^{\&\wedge}$ Granulomas, alveolar–bronchiolar junctions 9/3.4$^{\&\wedge}$ **13 wks recovery** Alveolar macrophages n=2/1.0$^{\&}$ Macrophage aggregations n=2/1.0$^{\&}$ Granulomatous inflammation n=5/2.8$^{\&\wedge}$ Granulomas, alveolar–bronchiolar junctions 5/3.4$^{\&\wedge}$ **52 wks recovery** Alveolar macrophages n=2/1.0$^{\&}$
3	Male rats (strain and number of animals unknown) / ECETOC, 2006; Becker et al. 2013/ Dow Corning (1972)	SAS-HMDS Doses (mg/m^3):0, 10, 50, 150 6 h/d, 5 d/wk, 12 months	✓Dose-related mortality was observed Control group (mortality 8%, no data on historical controls) 10 mg/m^3 (mortality 12%, no data on when mortality occurred), no other effects reported **50 mg/m^3 (mortality 26%) and 150 mg/m^3 (mortality 33%)** <u>Observations at surviving animals</u> White foci on lung surfaces and collections of foamy macrophages within the alveoli. Peribronchial lymph nodes enlarged Criteria for classification$^{\#}$ – inhalation STOT RE 2 ≤ 50 mg/m^3

4	Monkey, Cynomolgus Male (number of animals unknown) / ECETOC, 2006; Becker et al., 2013 / Dow Corning (1972)	SAS-HMDS Doses (mg/m^3):0, 10, 50, 150 6 h/d, 5 d/wk, 12 months	**10 mg/m^3** No effect. **50 mg/m^3 and 150 mg/m^3** Interstitial fibrosis not resolving or progressing during recovery. Peribronchial lymph nodes enlarged. Criteria for classification# – inhalation STOT RE 2 ≤ 50 mg/m^3
5	Rat, Sprague-Dawley Female n=80 / Becker et al., 2013 / Degussa (1962)	SAS-DDS One dose (mg/m^3): 50 5h/d, twice/wk, 8 or 12 months with 0-5 months recovery MMAD < 7µm	During exposure: Interstitial white dust deposits slightly enlarged lymph nodes Increased number of granular phagocytes Local fibrosis. Post recovery period: Interstitial grey-white dust deposits (increasing at 5 months) Moderately enlarged grey-black lymph nodes (peak at 1 month, decreasing afterwards) Slight epithelial desquamation in the lung up to 1 month Locally perivascular, peribronchiolar dust cell deposits with slight to moderate formation of fibrous tissue Part of the alveolar wall thickening. Increased number of granular phagocytes and local fibrosis in lymph nodes (signs of recovery 1-5 months) Criteria for classification# – inhalation STOT RE 2 ≤ 125 mg/m^3
6	Rat, Wistar (10/sex) / ECETOC, 2006 / Wacker (1998)	SAS-HMDS HDK SKS300 Doses (mg/m^3): 0, 0.51, 2.05, 10.01 6 h/d, 5 d/wk, 13 wk	**10.01 mg/m^3** Lungs and tracheobronchial lymph nodes: significant increase in absolute/relative weight Lungs with red appearance/ white spot(s) on the lungs in females Alveolar macrophages accumulation with few polymorphonuclear cells, accompanied by bronchiolar-alveolar epithelial hyperplasia and interstitial inflammatory cell infiltrates in lungs. Increased histiocytosis in lung draining mediastinal lymph nodes Macrophage aggregates in paracortex and/or germinal centres. Statistically significant increases in total protein, LDH and NAG in lung lavage fluid.** No indication of increased birefringence (typical for interstitial fibrosis). Clear recovery of all effects. NOEL = 0.51 mg/m^3 Criteria for classification# – inhalation STOT RE 1 ≤ 20 mg/m^3

Oral studies			
7	Wistar rats (5 /sex) / A6.3.1 / Degussa (1964)	No guideline, no GLP study SAS-DDS Aerosil R972 Doses (mg/Kg bw): 0, 500, 1000, 2000 (increasing successively to 16000) 5 wk (8 wk high-dose group); 7d/wk	No lung effects were observed. Liver was the target organ due to the observed atrophy LOAEL = 1000 mg/kg bw/d Criteria for classification# – oral STOT RE 2 ≤ 300 mg/kg bw/d
8	Wistar rats (20/sex) / A6.4.1 / Degussa (1964)	No guideline, no GLP study SAS-DDS Aerosil R972 Doses (mg/Kg bw): 0, 500 6 months; 7d/wk	No treatment related effects were observed. Criteria for classification# – oral STOT RE 2 ≤ 50 mg/kg bw/d
9	Wistar rats (20/sex) / A6.5 / Degussa (1969)	No guideline, no GLP study SAS-DDS Aerosil R972 Doses (mg/Kg bw): 0, 100 24 months; 7d/wk	<u>Clinical signs</u> Signs indicative of chronic bronchopneumonia in 7 animals from each sex, accompanied with hyperplasia of peribronchial lymphoid tissue, enlarged bronchia and focal emphysema. It is stated in the CAR, that "the changes reported for the lung are known to occur with similar incidences in control animals and were therefore not treatment-related effects". However, no actual data on controls, historical controls or statistical analysis are available. Kidney effects, changes in the genital tract of females and fat deposits in both sexes were also no-treatment related according to the DS and CAR. Criteria for classification# – oral STOT RE 2 ≤ 12.5 mg/kg bw/d

** The literature references are to the review article where the studies are mentioned, as well as the source and year of the study*

& Severity grade 1-5 (Weber et al., 2018)

^ Statistically significant

 # Haber's rule applied

¥ From the necropsy at the end of treatment, only sections from males were available. Therefore, comparison is restricted only to males during the recovery period

** For studies #3-#6 a general description of the clinical signs is provided in Becker et al. (2013) "In rats, clinical signs included crusty eyes, muzzle, and nose; crust around ear tags; closed eyes; irregular breathing; irritable disposition; lacrimation and salivation; scabs; and red- and yellow-/brown-stained fur. At 2 weeks, there was an increase in lymphocytes and neutrophils. Reduced body weights were observed. Silica was deposited in the lungs and lymph nodes, but the deposits cleared over time."*

*** N-Acetyl-/β-glucosaminidase (NAG) is a high molecular-weight (~140 kDa) hydrolytic lysosomal enzyme*

that is found in many tissues of the body. It breaks chemical bonds of glycosides and amino sugars that form structural components in many tissues. It is necessary for the degradation and disposal of various parts of the cell, including the cell membrane.

In the 14-day inhalation study (A6.3.3) that served as a pilot to the 13-weeks OECD TG 413 comparable GLP compliant study (A6.4.3_01), all treated groups suffered from respiratory distress that escalated to moderate dyspnoea at the mid dose (87 mg/m^3). Nevertheless, there is a doubt whether respiratory distress was actually seen at the low dose (31 mg/m^3), as the data presentation in the CAR are confusing. Aging of the animals could not account for such a clinical finding. In addition, in the 13-week key inhalation study in the CLH report no particular clinical signs are reported in the CAR, while in Reuzel *et al*. (1991), which reviewed the original 13-week inhalation study it is stated that "*Respiratory distress was observed in all rats exposed to Aerosil R974*". On the other hand, all inhalation studies of the open literature used for classification purposes (studies #3-#6 in the Table above) reported irregular breathing as a consistent clinical sign. Histopathology reports showed mainly transient inflammation especially in the alveolar region, and local injury of the lungs and in some cases of the mediastinal lymph nodes and more rarely the nose. Some local inflammation is expected as an adaptive response to the inhalation of insoluble particles. Also, silica (measured as Si) was found to have been retained in the lungs of all exposed animals in a concentration-related manner and was also found in the tracheobronchial lymph nodes. Si levels in the lungs were decreased and the level in the lymph nodes increased, compared to the levels measured immediately after exposure (Wacker, 1998), indicating that SAS is most probably solubilized or effectively cleared to lymph node tissues, which also showed evidence of inflammation. The reported interstitial fibrosis and other serious adverse histopathological findings reported in the A6.4.3_01 study, became questionable in the light of the Weber *et al.* (2018) re-evaluation of the findings of the study. Following re-evaluation it was concluded that there was no fibrosis detected and that all effects appeared reversible within 13-52 weeks. RAC notes the following:

- the re-evaluation did not concern all animals, and only one lung section per animal;
- for re-evaluation, the almost 30-year old slides were de-cover-slipped, re-stained (with standard hematoxylin and eosin staining) and then cover-slipped again, whereby the de-cover-slipping may potentially have damaged the original tissue samples;
- the claimed recovery pertains to unusually long recovery periods for a 13-week rat study (13-52 weeks, as compared to 4 weeks as recommended in the OECD test guideline).

Moreover, interstitial fibrosis is also reported in the 1-yr study with monkeys (by Dow Corning (1972) (study #4, reviewed in Becker *et al.* (2013) and ECETOC, 2006) and which did not resolve during recovery, but very few study details are available; for example the number of animals, the incidence of observations and when during the study clinical signs and histopathological findings are observed are not known. It is also unclear if and at which dose irregular breathing, a potentially related clinical sign, is observed. The original results of the Dow Corning (1972) study are not available. In addition, in the Degussa (1962) study (study #5), reviewed in Becker *et al.* (2013), female rats treated for 8 or 12 months showed local fibrosis is reported at 50 mg/m^3, which persisted even during the recovery period. On the other hand in the 13-week rat study by Wacker (1998) (study #6), reviewed in ECETOC (2006), no indication of increased birefringence

(typical for interstitial fibrosis) was reported. However, histiocytosis in lung draining mediastinal lymph nodes was seen as adverse finding in this study, albeit reported to be reversible after a 13-week recovery period. Unfortunately, the original results of the Wacker (1998) study (rated as reliable guideline study by ECETOC) are not available.

Fibrogenesis, which is a reversible process, is proposed to be the main finding in the Weber *et al.* (2018) re-evaluation study instead of fibrosis, along with extensive local inflammation in the lung. Nevertheless, the increase of lung collagen content (the specific Van Gieson stain was not used in the re-evaluation nor was OH-proline was measured), the septal cellularity and the alveolar bronchiolisation originally reported in Reuzel *et al*. (1991) (not disputed by Weber *et. al.,* 2018 in its re-evaluation), are still present at least at the end of exposure and all point to tissue remodelling or injury. In addition, the high lactate dehydrogenase (LDH) and N-acetyl-beta-D-glucosaminidase (NAG) activity in the lung lavage fluid (ECETOC, 2006; Wacker, 1998) also supports tissue injury. These findings could account for exposure-related fibrogenesis and structural remodelling of the lung tissue, which are reversible but cannot be excluded as an adverse effect that could progress to fibrosis, if exposure persists and in the presence of another detrimental pathology, such as infection. In all cases, histopathological findings like these could account for clinical symptoms of respiratory distress.

The available oral repeated dose toxicity studies establish the absence of significant toxicity by this route of exposure. Dermal exposure is not expected to cause toxicity as silanamine is neither skin corrosive/irritant nor sensitiser and bioavailability via skin penetration is expected to be minimal.

According to the CLP regulation, STOT-RE is assigned on the basis of findings of 'significant' or 'severe' toxicity. In this context 'significant' means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. 'Severe' effects are generally more profound or serious than 'significant' effects and are of a considerably adverse nature which significantly impact on health.

In the case of silanamine (SAS-HMDS), the effective dose in the various studies presented in the Table above mostly point to classification in category 2, although in two studies (#1 and #6) category 1 could also be supported and in study #2 the effective dose is close to the cut-off for category 1. Regarding the effects observed, some alterations in pulmonary function (breathing) are consistent among the majority of the repeated dose inhalation studies with hydrophobic SAS. Hydrophobic SAS induced treatment-related effects reflecting inflammation of lung tissue (main mechanism of toxicity identified), associated with a morphological tissue reaction (hypertrophy, lung injury, partial hyperplasia of the bronchiolar epithelium, collagen remodelling). The vast majority of the effects disappeared during recovery, showing clear signs of reversibility. Only the inflammation effects could be regarded as adaptive (compensatory) changes, but the adversity of the consequences and the clinical toxicity (i.e. impaired breathing) upon cessation of exposure is still present. Given further remaining uncertainties on whether or not there was fibrosis in key study #2, RAC considers classification warranted. Based on a weight of the evidence of all available data, RAC supports the DS proposal for **classifying silanamine as STOT RE 2, H373 (lung, inhalation).**

10.13 Aspiration hazard

No study available.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Silicon dioxide occurs ubiquitously in the environment. It accounts for approximately 27.6% of the earth's crust and is widely distributed in water, soils and plant and animal tissues. Silicon dioxide is regarded as inert in all but extreme conditions.

Initially, the applicant Degussa notified two CAS numbers for pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide (CAS n°68909-20-6 and 68611-44-9). These silicon dioxides are a part of the wider synthetic amorphous silica family (CAS n°7631-86-9). These surfaces modified silica are obtained by reaction with hexamethylsilazane (Aerosil R 812 and R 812 S) or dimethyldichlorosilane (Aerosil R 972, R 974, R 976) which induces the fixation of methyl group on the surface of the molecule. By this surface modification, synthetic amorphous silica, which are originally hydrophilic, are rendered physico-chemically hydrophobic. These hydrophobic amorphous silica are therefore inorganic compounds with an organic carbon content of 0.6 – 4.0 % (w/w). Nevertheless more than 95 % of the hydrophobic amorphous silica is comprised of polymerically bound silicon dioxide (SiO_2). The majority of hydroxyl groups on the particle surface are covalently bound to dimethylsilyl groups (Aerosil types of the 97 series) or trimethylsilyl groups (Aerosil R 812 and R 812 S). Methylation results in highly hydrophobic solids which are very stable, insoluble in water and non-volatile. Degradation is only possible by physical means: e.g. combustion would result in >99.5 % silicon dioxide, small amounts of water and carbon dioxide. When released into the environment, these forms are expected to combine with soil or sediment organic matter and adopt the same behaviour as natural silica.

For these reasons, a reduced set of data was accepted in the frame of biocide assessment.

Moreover, following Biocide Technical Meeting II 2011, it has been decided that only Aerosil R 812 and R 812 S will be kept for the assessment of the active substance. Nevertheless, France noted that all environmental studies are performed with Aerosil R 972 and R 974. However, based on physico-chemistry data (see section 1 – Identity of the substance), Aerosil R 972 and R 974 are considered similar to Aerosil R 812 and R 812 S.

It has to be noted the substance is a nanoparticle, however the available studies are not designed to assess specifically ecotoxicity linked to this property.

11.1 Rapid degradability of organic substances

Biodegradation study is not applicable. Hydrophobic amorphous silica are inorganic compounds with an organic carbon content of 0.6 – 4.0 % (w/w). More than 95 % of the hydrophobic amorphous silica is formed of polymerically bound silicon dioxide (SiO_2). The majority of hydroxyl groups on the particle surface are covalently bound to dimethylsilyl groups (Aerosil types of the 97 series) or trimethylsilyl groups (Aerosil R 812 and R 812 S). Methylation results in highly hydrophobic solids which are very stable and insoluble in water and not accessible to biological transformation. The chemical structure and composition of these silica particles is of inorganic rather than of organic nature. Therefore, biodegradation is not reasonably applicable to such inorganic substances and, considering its high stability and inertness, the study is not required.

Considering that the hydrophobic amorphous silica are inorganic compounds with an organic carbon content of only 0.6 – 4.0 % (w/w) and that more than 95 % of the hydrophobic amorphous silica is comprised of polymerically bound silicon dioxide (SiO_2), even if the organic

part of the molecule was degraded, the metabolites formed would not exceed 4.0 %, which remains below the trigger of 10 %.

11.1.1 Ready biodegradability

Not relevant

11.1.2 BOD_5/COD

Not relevant

11.1.3 Hydrolysis

Hydrolysis study is not scientifically justified. Amorphous silica is rendered highly hydrophobic through blocking the polar superficial hydroxy groups by dimethylsilyl groups (Aerosil 972 or 974) or by trimethylsilyl groups (Aerosil 812 or 812S). This surface can be considered resistant to hydrolytic attack under environmental conditions and even under boiling in water at neutral pH. Therefore, based on the chemical nature (inorganic character, high chemical stability of the Si-O bond and very low solubility in water), no pH-dependent hydrolysis will occur in water at low and high temperatures.

11.1.4 Other convincing scientific evidence

No further available data

11.1.5 Field investigations and monitoring data (if relevant for C&L)

No available data

11.1.6 Inherent and enhanced ready biodegradability tests

Not relevant

11.1.7 Water, water-sediment and soil degradation data (including simulation studies)

No available data

11.1.8 Photochemical degradation

Photolysis in water and air

Photolysis studies in water and in air are not scientifically necessary.

Aerosils R 812, R 812 S, R 972, and R 974, typical representatives of pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide, are of inorganic nature and are insoluble in water. Furthermore, the compounds do not absorb light above 270 nm. Therefore, based on the physico-chemical nature (inorganic structure, chemical stability, i.e. high stability of the Si-O bond, absence of water solubility and lack of interference with light), no light-induced transformation is expected in water.

For the same reasons of physico-chemical nature of pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide, no photo degradation in air will occur. Moreover, the exposure via the atmospheric compartment is not considered relevant as the volatility of these compounds is negligible.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant

11.2.1 Summary of data/information on environmental transformation

Not relevant

11.3 Environmental fate and other relevant information

Hydrophobic amorphous silica are inorganic compounds with an organic carbon content of 0.6 – 4.0 % (w/w). More than 95 % of the hydrophobic amorphous silica is formed of polymerically bound silicon dioxide (SiO_2). Therefore, biodegradation is not reasonably applicable to such inorganic substances and, considering its high stability and inertness, the study is not required. Moreover, based on the chemical nature of the substance (inorganic character, high chemical stability of the Si-O bond and very low solubility in water), no pH-dependent hydrolysis will occur in water at low and high temperatures.

11.4 Bioaccumulation

The pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide is considered as inorganic substances composed by 95% of polymerically bound silicon dioxide (carbon organic content is less than 4%). These synthetic amorphous silica are practically insoluble in water and thus are barely bioavailable via the water phase.

Although highly hydrophobic, these synthetic amorphous silica do not dissolve in non-polar fluids or lipids in view of their stable solid structure. Hence, they lack the typical features of lipophilicity and lipid solubility. Moreover amorphous silicon dioxide does not have any intrinsic properties, which suggest that it will bioaccumulate in the environment.

In a weight of evidence approach, the overall information indicates a low potential for bioaccumulation.

11.5 Acute aquatic hazard

As the surface modified amorphous silica are hydrophobic, nearly insoluble (<1 µg/L) and complicated to analyse, with a limit of determination in water (1 mg/L) (see Doc IIIA section A4) higher than the solubility limit, these substances are difficult to test according to the standard ecotoxicity guidelines. The studies carried out with higher concentrations than the solubility limit were considered acceptable in the frame of biocide assessment even in absence of analytical measurement, taking into account of the high stability of the molecule. Moreover, the substance tested at high dose rate, up to 10 000 mg/L, showed no toxicity to aquatic organisms as demonstrated hereafter.

These issues were discussed and agreed during Biocide Technical Meeting III10. Indeed, in general cases, the results should be treated as invalid. Nevertheless, the studies can be used in a weight of evidence to show that there are no effect on aquatic organisms. Indeed, due to the large excess of the substance in studies, it was considered that its solubility limit was achieved during the tests. The substance shows no effects on aquatic organisms even at the high concentration tested (1 000 to 10 000 mg/L).

The pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide was tested on environmental organisms under different commercial forms: Aerosil R 974 for acute fish and daphnia, Aerosil R 972 for algae and Aerosil R 812S for microorganisms. The subjects of the Biocidal Product Dossier relevant for the claimed application are reaction products of synthetic

amorphous silica after treatment with hexamethylsilazane (CAS 68909-20-6; Aerosil R 812 and Aerosil 812S) or dimethyldichlorosilane (CAS 68611-44-9; Aerosil R 972, Aerosil R 974 and Aerosil R 976). Whatever the reactant used (hexamethylsilazane or dimethyldichlorosilane) the aim of the modification is to block the silanol group of the molecule in order to render the material hydrophobic. The chemical groups added by the reaction have no particular activity by themselves. The main variation between the different types of Aerosil is their surface area conferring to them some different rheological properties necessary for their commercial use. In view of these data, as indicated in the introduction of environmental hazards, France has considered that results from aquatic studies could be extrapolated from one type of Aerosil to another in the frame of biocide assessment (see also section 1 – Identity of the substance).

Table 11.5.1: Summary of relevant information on acute aquatic toxicity

Method	Species	Exposure	Results[1]			Remarks	Reference
			LC_0	LC_{50}	LC_{100}		
OECD 203 (1984), GLP RI : 2	*Brachydanio rerio*	Static / 96h	>10000 mg/L	>10000 mg/L	>10000 mg/L	Substance tested: Aerosil R 974	Hooftman RN and van Drongelen-Sevenhuijsen D (1992a)
OECD 202 (1984), GLP RI : 2	*Daphnia magna*, immobilisation	Static / 48h	>10000 mg/L	>10000 mg/L	>10000 mg/L	Substance tested: Aerosil R 974	Hooftman RN and van Drongelen-Sevenhuijsen D (1992b)
OECD 201 (1984), GLP RI : 2	*Scenedesmus subspicatus* Biomass and growth	Static / 72h	>10000 mg/L	>10000 mg/L	>10000 mg/L	24-h water extract of Aerosil R 972	Lebertz H (1999)

[1] Concerning the expression of the endpoints, normally the rule is to set the LC_{50} to the solubility limit. Based on the physico-chemical properties of the test substance which is practically insoluble in water, the result obtained for the acute toxicity tests expressed in nominal concentrations was accepted for biocides risk assessment purposes.

11.5.1 Acute (short-term) toxicity to fish

Aerosil R 974 was tested on Zebrafish (*Brachydanio rerio*) in static system during 96 h. The purity of the technical substance was 100%. The nominal test concentrations were a control, 1 000 and 10 000 mg/L.

Some deviations to OECD Guideline 203 have to be reported. Temperature was slightly higher than recommended range for the species used. There was no indication on fish acclimatising before the assay. There was no analytical measurement of the actual test concentrations. Non-dissolved substance was not separated and removed before testing.

Whatever the nominal concentrations tested, no mortality and no sublethal effect occurred. Based on nominal concentrations of test substance, the LC_{50} value was > 10 000 mg/L. However, LC_{50} could not be defined in actual concentration. Thus, it can be concluded that the test substance has a low acute toxicity to the test organism *Brachydanio rerio*.

Please refer to Doc IIIA section 7.4 for further details.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Aerosil R 974 was tested on *Daphnia magna* in static system during 24 h. The purity of the technical substance was 100%. The nominal test concentrations were a control, 1 000 and 10 000 mg/L. As hydrophobic amorphous silicate is nearly insoluble, test suspensions were stirred in test vessels for about 20 h. All the concentrations were tested non-filtered. The 10 000 mg/L concentration was also tested after a filtration through a wad of perlon wool.

No analytical measurement of test concentrations was performed. The determination of LC_{50} could be made only on the nominal concentrations of test substance.

Whatever the group tested, no immobilisation was observed after 24h, and the EC_{50} was estimated to be > 10 000 mg/L (nominal concentration). It was concluded that the test substance was not acutely toxic to test organisms within its aqueous solubility. Extrapolation to the standard test duration of 48 h appears to be justified as no adverse effect were observed at 24 h with high loading of the test compound taking also into account the insolubility of the substance.

Please refer to Doc IIIA section 7.4 for further details.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

Effects of Aerosil R 972 on algal growth were evaluated with a 72 hour toxicity test in a freshwater algae *Scenedesmus subspicatus*, in static conditions, at the nominal concentrations: 0, 100, 1 000 and 10 000 mg/L. Test suspensions were incubated in a shaking machine for 24 hours and then filtered. Eluates were used for the test. No analytical measurement of test concentrations was performed. The determination of LC_{50} could be made only on the nominal concentrations of test substance.

Cell concentration in control cultures increased at least by a factor 16 within 3 days. No reduction in growth rate was observed in treated group after 72h. A reduction of biomass production of 1.5% was observed only at 100.8 mg/L after 72 h. Therefore, EbC_{50} and ErC_{50} were estimated to be > 10 000 mg/L (nominal concentration). An important pH deviation in the control cultures and test vessels (about 3 units) without explanation is observed but does not discredit the study results.

The test substance does not inhibit the growth of the freshwater algae *Scenedesmus subspicatus* within its aqueous solubility.

Please refer to Doc IIIA section 7.4 for further details.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No other available data

11.6 Long-term aquatic hazard

No available data

11.6.1 Chronic toxicity to fish

No available data

11.6.2 Chronic toxicity to aquatic invertebrates

No available data

11.6.3 Chronic toxicity to algae or other aquatic plants

No available data

11.6.4 Chronic toxicity to other aquatic organisms

No available data

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

In the frame of Biocide Regulation, only acute toxicity tests for pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide were provided and accepted for risk assessment purpose. All available acute L(E)C_{50} values for all three trophic levels are >1 mg/L. Despite the low reliability of these tests, due to the high insolubility of the substance and the lack of measurement concentrations, no effect was observed in the ecotoxitity tests at the hogh loading rate.

Therefore, based on the available information, no classification with Aquatic Acute 1 is necessary.

➔ **No classification**

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

No chronic studies are available for pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide for any of the three trophic levels. Therefore acute toxicity tests should be used following the Figure 4.1.1 of the Guidance in the Application of the CLP Criteria – version 5.0 – July 2017. No effect was observed in the acute ecotoxitity tests performed under tested conditions at the nominal concentration of 1000 and 10000mg/L.

Weight of evidence indicating low potential to bioaccumulate.

The conventional biodegradation studies designed to test organic substances are not reasonably applicable for such inorganic substances considering its high stability and inertness. Amorphous silica is considered as no rapidly degradable.

Therefore, based on these consideration, no classification with Aquatic chronic is necessary.

➔ **No classification**

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

No classification for hazards to the aquatic environment is proposed for pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS in the evaluation of the aquatic hazard stated that surface-modified synthetic amorphous silica, which are originally hydrophilic, are rendered physico-chemically hydrophobic. These hydrophobic amorphous silica are therefore inorganic compounds with an organic carbon content of 0.6 – 4.0% (w/w). More than 95% of the hydrophobic amorphous silica is comprised of polymerically bound silicon dioxide (SiO_2). The majority of hydroxyl groups on the particle surface are covalently bound to either dimethylsilyl groups (SAS-DDS) or trimethylsilyl groups (SAS-HMDS). Methylation results in highly hydrophobic solids, which are very stable, insoluble in water and non-volatile. Degradation is only possible by physical means: e.g. combustion would result in >99.5% silicon dioxide, small amounts of water and carbon dioxide. When released into the environment, these forms are expected to combine with soil or sediment organic matter and adopt the same behaviour as natural silica. The DS added:

Biodegradation

The highly hydrophobic surface modified SAS are very stable and insoluble in water and not accessible to biological transformation. The chemical structure and composition of these silica particles is of inorganic rather than of organic nature and consequently no biodegradation is expected.

Hydrolysis

The surface of the hydrophobic SAS can be considered resistant to hydrolytic attack under environmental conditions and even under boiling in water at neutral pH. Therefore, based on the chemical nature (inorganic character, high chemical stability of the Si-O bond and very low solubility in water), no pH-dependent hydrolysis will occur in water at low and high temperatures.

Photolysis in water and air

The hydrophobic SAS compounds do not absorb light above 270 nm. Therefore, based on the physico-chemical nature (inorganic structure, chemical stability, i.e. high stability of the Si-O bond, absence of water solubility and lack of interference with light), no light-induced transformation is expected in water.

For the same reasons of physico-chemical nature of pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide, no photo degradation in air will occur. Moreover, the exposure via the atmospheric compartment is not considered relevant, as the volatility of these compounds is negligible.

Bioaccumulation

The hydrophobic SAS are considered inorganic substances composed by 95% of polymerically bound silicon dioxide (carbon organic content is less than 4%). These synthetic amorphous silica are practically insoluble in water and thus are barely bioavailable via the water phase. In addition, although highly hydrophobic, these synthetic amorphous silica do not dissolve in non-polar fluids or

lipids in view of their stable solid structure. Hence, they lack the typical features of lipophilicity and lipid solubility. Moreover, amorphous silicon dioxide does not have any intrinsic properties, which suggest that it will bioaccumulate in the environment. Thus, the DS stated that in a weight of evidence approach, the overall information indicates a low potential for bioaccumulation.

Aquatic Hazard

There are only studies for aquatic acute toxicity.

The DS proposed no classification for hazards to the aquatic environment for silanamine (SAS-HMDS) based on three acute studies in fish, *daphnia* and algae with the read across substances SAS-DDS. The acute L(E)C_{50} values for all three trophic levels were above 1 mg/L at nominal concentrations above 10000 mg/L. Despite the low reliability of these tests, due to the high insolubility of the substance in combination with the lack of analytical measurement concentrations, no physical and chemical effects were observed in the aquatic toxicity tests, even at a very high loading rate. A more comprehensive analysis of the aquatic acute toxicity studies will follow in the Assessment and comparison with the classification criteria section of the opinion.

Comments received during public consultation

There were three MSCA comments regarding the environmental hazard evaluation during public consultation.

The first MSCA focused on the lack of a robust analysis and justification for the read across for the environmental hazards. The MSCA noted that the read across justification was only based on physico/chemical characteristics of the substances (particle size, coating etc.) and not on aspects like toxicity, fate and toxicokinetics. However, the MSCA added that the ecotoxicity endpoints for the read across substance are > 10000 mg/L, these values are far above any trigger for environmental classification. The MSCA concluded that when a more proper and more robust scientific justification is provided, then the proposal for no classification for environmental hazards would be accepted by the MSCA.

The second MSCA questioned the reliability of the available aquatic toxicity studies and is of the opinion that the studies are inadequate and invalid for classification purpose of this nanomaterial. This opinion was based on the following observations:

- The protocol for testing of poorly soluble substances was not followed. Analytical measurements of exposure concentrations were not determined and as a result the maximum dissolved concentrations could not be validated.
- Although hydrophobic SAS are produced as nanomaterials, the protocol for nanomaterial testing was not followed.

The third MSCA stated that although it does not envisage silicon dioxide to present a bioaccumulation hazard under normal circumstances, the bioavailability and uptake of these nanoparticles (which have been intentionally surface modified to affect their hydrophobicity) might well be different or operate through different mechanisms and timescales. In addition, the MSCA noted that in general it is uncomfortable with substances manufactured to be biologically active, such as biocides and pesticides, not even having a 'safety net' environmental classification. Thus, the MSCA proposed a Chronic Category 4, H413. Lastly, the MSCA added that testing specific to nanoparticles has not been conducted, although OECD test guidelines are in development.

Additional key elements

There are no additional studies in the open literature but the ecotoxicological properties of hydrophobic SAS have been reviewed by (Pölloth, 2012; EPA, 2011; ECETOC, 2006 and OECD SIDS, 2004). A short summary is shown below.

Environmental Fate: Under normal environmental conditions, silicon dioxide is an inert substance with no known degradation products. At ambient temperature and pH, hydrophobic SAS are practically insoluble in water. Due to the known tendency to supersaturate, solubility and dissolution rates are an important parameters to consider. Dissolution rates at ambient conditions are very low and similar to the solubility levels. SAS are not volatile and have no lipophilic character. SAS will therefore settle mainly into soils/sediments and weakly into water. SiO_2 is expected to combine indistinguishably with the soil layer or sediment due to the chemical similarity with inorganic soil matter (OECD SIDS, 2004). No adsorption of humic acids was observed on nano-sized SiO_2, either in the spherical- or in the porous-form (Pölloth, 2012). Bioavailable forms of silica are dissolved silica [$Si(OH)_4$], silicic acid and silicates. Silicates are found throughout the Earth's lithosphere. Based on the chemical nature of silica and silicates (inorganic structure and chemical stability of the compound: Si-O bond is highly stable), no photo- or chemical degradation is expected (OECD SIDS, 2004). Biodegradation and speciation of SiO_2 (e.g., dissociation or complexation) will not occur in aquatic media under normal conditions, though particle size may change due to aggregation and agglomeration. Due to its inherent physico-chemical properties, such as the absence of lipophilicity as well as the capability of organisms to eliminate absorbed SiO_2 components, bioaccumulation is not to be expected (Pölloth, 2012).

Ecotoxicity: No effects were found in the acute aquatic toxicity studies with surface treated SAS (EPA, 2011). The studies used in the evaluation were the same ones described in the CLH report. With regard to chronic aquatic toxicity data, the open literature reviews concluded that although there were no chronic aquatic toxicity data for SAS, there is no evidence of harmful long-term effects due to the known inherent physico-chemical properties, absence of acute toxic effects as well as the ubiquitous presence of silica and silicates in the environment.

In conclusion, there is no evidence of significant acute toxicity of SAS to organisms in the environment. SAS did not exhibit toxicity when tested on aquatic organisms under laboratory conditions. On a global scale, the level of man-made SAS represents up to 2.4% of the dissolved silica naturally present in the aquatic environment. The rate of SAS released into the environment during the product life cycle is negligible in comparison with the natural flux of silica in the environment (ECETOC, 2006). Thus, hydrophobic SAS presents a low risk of adverse effects in the environment.

Assessment and comparison with the classification criteria

RAC agrees with the DS' analysis regarding degradation, hydrolysis, photolysis in water and air, and bioaccumulation. The hydrophobic amorphous silica are very stable and insoluble in water and not accessible to biological transformation. These substances are not expected to rapidly degrade, hydrolyse or bioaccumulate. In relation to degradation, RAC adds that the organic coating of the hydrophobic SAS could make these substances more susceptible to both biotic and abiotic degradation as compared with the non-treated SAS, but still there is no data to support this hypothesis. In addition, the organic moiety is a small part of the substance (carbon content <5%)

to trigger rapid degradability. However, it should be noted that no data is available for rapid removal of SASs from the water column, a test more relevant than rapid degradability for these type of substances. In addition, regarding bioaccumulation, although SAS are not expected to significantly bioaccumulate, based on their chemistry and their biogeochemical cycle in nature, there is no actual data to unequivocally support it, especially since the methodologies for the testing of nanomaterials have not yet been finalized.

Acute aquatic toxicity

Table: *Summary of relevant information on acute aquatic toxicity*

Method	Species	Exposure	Results LC_0	Results LC_{50}	Results LC_{100}	Test material	Reference
A7.4.1.1 OECD TG 203 GLP Guideline study with acceptable restrictions (ECETOC)	*Brachydanio rerio* mortality	Static 96h	>10000 mg/L	>10000 mg/L	>10000 mg/L	SAS-DDS (Aerosil R974)	Anonymous (1992a)
A7.4.1.2 OECD TG 202 GLP Guideline study with acceptable restrictions (ECETOC)	*Daphnia magna*, immobilisation	Static 48h	>10000 mg/L	>10000 mg/L	>10000 mg/L	SAS-DDS (Aerosil R974)	Hooftman and van Drongelen-Sevenhuijsen (1992b)
A7.4.1.3 OECD TG 201 GLP Guideline study with acceptable restrictions (ECETOC)	*Scenedesmus subspicatus* Biomass and growth	Static 72h	>10000 mg/L	>10000 mg/L	>10000 mg/L	SAS-DDS (Aerosil R972)	Lebertz (1999)

Acute Toxicity to Fish

Acute toxicity to fish was tested on zebrafish (*Brachydanio rerio*) in a static system for 96 h with SAS-DDS (Aerosil R974). The purity of the substance was 100%. The nominal test concentrations were a control, 1000 and 10000 mg/L. Test suspensions were stirred in test vessels for about 20 hours on a magnetic stirrer at 25°C and then allowed to stand for 4 hours. It is apparent that the ecotoxicity concentrations are loading rates rather than actual concentrations.

Several deviations to OECD TG 203 have to be reported. Temperature was slightly higher (25.4°C -

25.8°C) than the recommended range for the species used. There was no indication of fish acclimatising before the assay. The preparation of the solution for poorly soluble test substances was questionable because it was reported that all test solutions were turbid with dry test substance on the surface. According to the OECD Guidance document for difficult substances and mixtures, a 48-hour period for stirring is recommended to achieve the maximum dissolved concentration and non-dissolved substance should be separated and removed before testing which was not done in this case.

In addition, based on the physico-chemical properties of the test substance which is practically insoluble, it is evident that the concentration of the dissolved substance was not 80% of the initial concentration during the test and consequently, in accordance with the OECD TG 203, it is usually not possible to use the nominal concentrations for the calculation and reporting of the results. Moreover, the static-renewal, or flow-through exposure systems for poorly soluble compounds were also not followed.

However, mortality of control animals (<10%), concentration of dissolved oxygen in all test vessels (> 60% saturation), pH and weight and size of fish tested were in accordance to the OECD TG 203 validity criteria.

In conclusion, although no analytical measurement of substance test concentrations were performed, as no mortalities and no sub-lethal effects occurred in all the nominal concentrations tested, the test substance is presumed not to be acutely toxic to the test organism *Brachydanio rerio* within its aqueous solubility (LC_{50} > 10000 mg/L).

Acute Toxicity to Aquatic Invertebrates

Acute toxicity to aquatic invertebrates was tested on *Daphnia magna* in a static system for 24 h with SAS-DDS (Aerosil R974). The purity of the substance was 100%. The nominal test concentrations were a control, 1000 and 10000 mg/L. As hydrophobic amorphous silicate is nearly insoluble, test suspensions were stirred in test vessels for about 20 h. All the concentrations were tested non-filtered. The 10000 mg/L concentration was also tested after a filtration.

No effects were seen on immobility and no abnormal behaviour was noted on the test organisms after 24 hours. It was not possible to determine EC_{50} or NOEC values, as no adverse effects were observed in the doses tested. It was therefore concluded that the test substance was not acutely toxic to the test organism within its aqueous solubility.

There were several deficiencies in the test. The preparation of the solution for poorly soluble test substances is questionable because it is reported that "*all test solutions were turbid with dry test substance on the surface and/or on the bottom*". According to the OECD Guidance document for difficult substances and mixtures, a 48-hour period for stirring is recommended to achieve the maximum dissolved concentration and non-dissolved substance should be separated and removed before testing. Even in the case where the solution was filtered, dry substance remained on the surface of the test solution.

In addition, based on the physico-chemical properties of the test substance, which is practically insoluble, it is evident that the concentration of the dissolved substance was not 80-120% of the initial concentration during the test and consequently, in accordance with the OECD TG 202, it is not possible to use the nominal concentrations for the calculation and reporting of the results. Moreover, the static-renewal, or flow-through exposure systems recommended for poorly soluble compounds were also not used.

In conclusion, it should be noted that as no analytical measurement of test concentrations has been

performed and considering the deficiencies reported on the method of test media preparation, there is a risk of underestimating the toxicity. However, as neither immobility nor abnormal behaviour have been recorded in all nominal concentrations tested, the test substance is presumed not acutely toxic to the test organism *Daphnia magna* within its aqueous solubility (EC_{50} > 10000 mg/L, nominal concentration).

Acute Toxicity to Algae/Other aquatic plants

Acute toxicity on algal growth was tested on a freshwater algae (*Scenedesmus subspicatus*) in a static system for 72 h with SAS-DDS (Aerosil R972). The purity of the substance was 99.9%. The nominal test concentrations were a control, 100, 1000 and 10000 mg/L. As hydrophobic amorphous silicate is nearly insoluble, test suspensions were incubated in a shaking machine for 24 hours and then filtered. Eluates were used for the test. No analytical measurement of test concentrations was performed. The determination of LC_{50} could be made only on the nominal concentrations of the test substance.

Cell concentration in control cultures increased at least by a factor of 16 within 3 days. In the treated groups, no reduction in growth rate was observed after 72 hours. In the treated groups, an inhibition of biomass production of 1.5% was observed at 100.8 mg/L after 72 hours. No reduction in biomass was observed in the other treatments: at 1008 and 10000 mg/L, an increase of the biomass production of 0.8% and 7.2% was calculated, respectively.

There are deficiencies with the absence of an explanation for a pH deviation (about 3 units) and the absence of the results on the cell concentration for each flask at each measuring point with the variation coefficient for replicates of controls and test concentration. However, these reported deficiencies are considered of limited importance for the outcome of the study.

Thus, the test substance is presumed to not be acutely toxic to algae and does not inhibit the growth of the freshwater algae *Scenedesmus subspicatus* within its aqueous solubility (E_rC_{50} > 10000 mg/L, E_bC_{50} > 10000 mg/L, nominal concentration).

RAC recognises that there are several significant deficiencies in the studies regarding the evaluation of the environmental hazards.

- There are no studies with SAS-HMDS, only with the read across substances which have a slightly different surface coating. However, the read-across justification is supported by RAC and explained in the respective section of the opinion;
- The actual exposure concentrations of the substances were not measured in the available studies for the three trophic levels. However, it is noted that the nominal concentration of > 10000 mg/L is considerably higher than the value for triggering classification and much higher than the solubility of the material in water. The test media remained turbid throughout the test, indicating that the limit of solubility of the product was exceeded. The analytical monitoring and other test conditions were not protocol-compliant. Moreover, the protocol for poorly soluble substances was not followed;
- Although hydrophobic SAS are produced as nanomaterials, the protocol for nanomaterial testing was not followed. Low solubility versus dissolution rates, acute versus chronic testing are key aspects which are not discussed in the CLH dossier and data is not available.

In conclusion, the hydrophobic surface modified amorphous silica are nearly insoluble in ambient temperature (< 1 mg/L) and difficult to test according to standard aquatic toxicity test guidelines. The studies carried out with higher concentrations than the solubility limit had significant deficiencies and the protocol for nanomaterials was not followed. Thus, as explained above, it is rather unlikely that SAS-HMDS would cause an acute hazard to aquatic organisms and the results from the available studies do not meet the CLP criteria. Consequently, **RAC proposes no classification for aquatic acute hazard due to insufficient data.**

Aquatic chronic toxicity

No chronic studies are available for hydrophobic SAS for any of the three trophic levels. Therefore acute toxicity tests should be used following Figure 4.1.1 of the CLP Regulation. No effect was observed in the acute ecotoxicity tests performed under tested conditions at the maximum nominal concentration of 10000 mg/L. Based on a weight of evidence approach, SAS-HMDS has a low potential to bioaccumulate. Moreover, the conventional biodegradation studies designed to test organic substances are not reasonably applicable for such inorganic substances considering its high stability and inertness. Amorphous silica is not considered rapidly degradable in general but the surface treated SAS could exhibit degradability due to the trimethyl/dimethyl coating. However, there still is no data to support this hypothesis.

Therefore, as in the aquatic acute endpoint and based on all of the above, RAC proposes **no classification for aquatic chronic toxicity due to insufficient data.**

Safety net classification

Regarding the biocidal activity of SAS-HMDS, RAC recognizes that its mode of action (sorptive or abrasive) is based on the functional impairment or destruction of the lipid-wax layer cuticle, which renders the animal unprotected from water loss and, as a result, could affect both aquatic and terrestrial organisms after chronic exposure. However, this was not confirmed in the available acute aquatic toxicity tests, as described above.

However, according to the CLP regulation, the safety net classification, chronic hazard category 4, is appropriate in cases when data do not allow classification based on the CLP criteria but there are nevertheless some grounds for concern. This includes, for example, poorly soluble substances for which no acute toxicity is recorded at levels up to the water solubility, and which are not rapidly degradable and have an experimentally determined BCF ≥ 500 (or, if absent, a log K_{ow} ≥ 4), indicating a potential to bioaccumulate. These substances will be classified in this category unless other scientific evidence exists showing classification to be unnecessary.

SAS-HMDS is a poorly soluble compound for which no acute toxicity is recorded (although with insufficient data), not rapidly degradable (although probably more degradable than hydrophilic SAS) but also not bioaccumulative. RAC recognises that the afore-mentioned criteria are indicative and not restrictive but RAC also notes that in this case only two out of the three criteria are met. In addition, adsorption to organic matter of sediment could limit the availability and reactivity of silanamine particles for aquatic and benthic organisms.

Thus, in a weight of evidence approach, considering the biocidal activity of SAS-HMDS, its mode of action, the suggested criteria for aquatic chronic 4 classification and the fact that SAS-HMDS is not bioaccumulative, RAC concludes that a safety net classification for SAS-HMDS is not warranted.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not relevant

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

Not relevant

12.1.2 Comparison with the CLP criteria

Not relevant

12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Not relevant

13 ADDITIONAL LABELLING

Although the mechanism of biocidal action of pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide is currently not clear, "*The Manual of Decisions for Implementation of Directive 98/8/EC Concerning the Placing on the Market of Biocidal Products*" updated on 10th July 2008 states in its section 2.3.3 that product containing amorphous silica in a water base *"seems to act through absorption of the lipid layer covering insect's chitin protection, which then leads to desiccation and death of the target organism".* By destroying the natural water barrier, the waxy layer of the cuticle and hence disrupting the functioning of the water preservation mechanism, silica interferes with physiological processes.

Therefore, considering the mode of action of the active substance, a labelling EUH 066: Repeated exposure may cause skin dryness or cracking, is proposed.

Additional Labelling

Summary of the Dossier Submitter's proposal

The DS proposed to label silanamine with the EUH066 "Repeated exposure may cause skin dryness or cracking" phrase based on the generally accepted but not proven mode of action for SAS-HMDS.

The mode of action of silanamine as a biocidal active substance has been clearly described in the CAR. More specifically, the insects are deprived of their functional water barrier (desiccation effect) due to the functional impairment or destruction of the lipid-wax layer cuticle. In general, there are two mechanisms with SAS identified:

- Sorptive dusts primarily act through adsorption to the exoskeleton of the insects and absorption of lipid contained in the outmost layer of the epicuticle;
- Abrasive dusts act through mechanical grinding and abrasion of the insects' wax layer lipids of

the wax layer of the insect's cuticle become enriched by the silica dust during treatment, while the wax layer becomes reduced. The hydrophobic character of the silica intensifies adsorption to the insect's surface. Hydrophobic SAS are believed to act as abrasive dusts and are also proven more effective.

The mode of action is relevant to the human skin surface. A layer of lipids, which are of both sebaceous and keratinocyte origin, covers the surface of the skin.

Studies or occupational exposure / epidemiological data on human skin exposed to hydrophobic SAS are not available. Repeated exposure to precipitated SAS (without personal protection) may cause mechanical irritation of the eye and drying/cracking of the skin (Plunkett and DeWitt, 1962; ECETOC, 2006).

Comments received during public consultation

No comments received.

Assessment

Based on the above, RAC considers that there is relevant evidence concerning the effects of hydrophobic SAS on the skin (Annex II 1.2.4 of the CLP) and therefore proposes **labelling with the EUH066: Repeated exposure may cause skin dryness or cracking**.

14 REFERENCES

References are listed in the Competent Authority report.

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15 ANNEXES

Documents IIIA