Committee for Risk Assessment

RAC

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

Adopted

5 December 2019

5 December 2019

CLH-O-0000006735-67-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical 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**

The proposal was submitted by **France** and received by RAC on **17 December 2018.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

France has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **4 March 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **3 May 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Nikolaos Spetseris**

Co-Rapporteur, appointed by RAC: **Christina Tsitsimpikou**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **5 December 2019**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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 Current Annex VI Entry										
Dossier submitters proposal	TBD	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)	EUH 066		
RAC opinion	TBD	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	Acute Tox. 2 STOT RE 2	H330 H373 (lungs, inhalation)	GHS06 Dgr	H330 H373 (lungs, inhalation)	EUH066	ATE = 0.45 mg/L (dusts or mists)	
Resulting Annex VI entry if agreed by COM	TBD	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	Acute Tox. 2 STOT RE 2	H330 H373 (lungs, inhalation)	GHS06 GHS08 Dgr	H330 H373 (lungs, inhalation)	EUH066	ATE = 0.45 mg/L (dusts or mists)	

GROUNDS FOR ADOPTION OF THE OPINION

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 in the Background Document).

Therefore, RAC has used **only the hydrophobic polymorph for classification purposes**.

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**.

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.

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 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)	> 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 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	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

	Positive control rats were treated with 0.6 mg silica (quartz DQ12; particle diameter 0.9 µm) **SAS-HMDS (Aerosil R812S)**	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**.

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.

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**.

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.**

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.**

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 Guinea pigs / A6.1.5 / 1984	OECD TG 406 GLP Reliability: 1 24/sex Silanamine (SAS-HMDS) A separate test was conducted with di-nitro-chloro benzene and was positive	Induction: The test substance was applied as a 0.1% dilution in physiological saline and propylene glycol (1:1) for all 3 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.	None of the treated animals showed a positive reaction. Neither mortality nor clinical symptoms of toxicity were 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.**

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.

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 to deaths) 14 days, 6h/d, 5d/wk	✓ 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 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		Revised histopathological observations **End of exposure, Males ¥** 10 animals Alveolar macrophages n=10/2.7&^ Macrophage aggregations n=10/1.4&^

			Pneumocyte type II hyperplasia n=9/1.9[&^] Granulomatous inflammation n=10/3.5[&^] Granulomas, alveolar–bronchiolar junctions 9/3.4[&^] **13 wks recovery** Alveolar macrophages n=2/1.0[&] Macrophage aggregations n=2/1.0[&] Granulomatous inflammation n=5/2.8[&^] Granulomas, alveolar–bronchiolar junctions 5/3.4[&^] **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 <u>One dose (mg/m^3): 50</u> 5h/d, twice/wk, 8 or 12 months with 0-5 months recovery MMAD < 7µm	<u>During exposure</u>: Interstitial white dust deposits slightly enlarged lymph nodes Increased number of granular phagocytes Local fibrosis. <u>Post recovery period</u>: 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).**

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.

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 Sampling time: 3d, 21d and 90d SAS-HMDS (Aerosil R812S)	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 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**

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 20/sex / 1969 / A6.7	No guideline. The study was conducted in 1969; at that time, 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)	Males/Females: At the end of the experimental period, the body weight of the treated animals was within the range of 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.**

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.

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	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

	histopathology were done on the pups The findings were not assessed statistically. The test substance was not defined in terms of purity.	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. Deficiencies:	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 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

	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.	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**.

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**.

ENVIRONMENTAL HAZARD EVALUATION

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.

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			Test material	Reference
			LC_0	LC_{50}	LC_{100}		
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.

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ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).

Annex 3 Records of the targeted public consultation in relation to the classification of acute toxicity