Committee for Risk Assessment

RAC

Opinion
on the harmonised classification and labelling
at EU level of

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

Pursuant to Article 77(3)(c) of Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals

EC Number: 272-697-1
CAS Number: 68909-20-6

A77-O-0000007327-71-01/F

Adopted

8 June 2023

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8 June 2023

A77-O-0000007327-71-01/F

Request of the European Commission to review the harmonised classification of silanamine as adopted by RAC in its opinion of 5 December 2019

Pursuant to Article 77(3)(c) of Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (the REACH Regulation), the Committee for Risk Assessment (RAC) has adopted an opinion on the harmonised Classification and labelling of:

Chemical name:	**Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica**
EC Number:	**272-697-1**
CAS Number:	**68909-20-6**

I PROCESS FOR ADOPTION OF THE OPINION

Following a request from The European Commission dated 26 September 2022, the Executive Director of ECHA in the mandate of 7 October 2022, requested RAC to prepare an opinion in relation to the harmonised classification of Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Michal Martínek**

Co-Rapporteur, appointed by RAC: **Benjamin Piña**

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 **8 June 2023** by **consensus**.

II Opinion of RAC

Background to the request

On 5 December 2019, RAC adopted an opinion on silanamine, 1,1,1-trimethyl-N- (trimethylsilyl)-, hydrolysis products with silica; pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide (HMDZ (hexamethyldisilazane) -treated SAS; EC Number 272-697-1).

The dossier submitter (France) had proposed a classification for Specific Target Organ Toxicity Repeated Exposure in category 2 (STOT RE 2) for effects on the lung by inhalation, but did not propose classification for acute toxicity. RAC, taking into account also a number of additional studies from the open literature and applying a weight of evidence approach, considered that the forms of hydrophobic, synthetic amorphous silica (SAS) described in the opinion have an acute inhalation effect in the rat. A key study with DDS (dichlorodimethylsilane) -treated SAS (Anonymous 1994a), the results of which RAC considered to be relevant also for HMDZ-treated SAS, gave an LC_{50} of 0.45 mg/L and led RAC to conclude that the substance should, in addition, be classified for acute toxicity by inhalation in category 2, with an ATE of 0.45 mg/L.

During the targeted consultation on the inhalation studies from the open literature, stakeholders had commented that the observed lethality was thought to be due to suffocation caused by the tendency of SAS to agglomerate, and thus a purely physical effect. RAC considered this aspect in the background document to the opinion but stated that no findings supporting this mechanism had been reported in the studies, and that histopathological examinations point to acute respiratory distress syndrome rather than to suffocation. Following adoption and publication of the RAC opinion, manufacturers of the substance provided an additional study which examined the mechanism for the observed acute toxicity of HMDZ-treated SAS via the inhalation route. In the view of the submitters, the study confirms a physical obstruction of the upper respiratory tract by agglomerated HMDZ-treated SAS as the cause of death by suffocation. They further considered that this effect cannot be extrapolated to humans and, as a physical effect, does not fulfil the criteria for a classification for acute toxicity via inhalation. The submitted information comprises a study report from a new GLP-compliant acute toxicity inhalation study, which was performed according to OECD TG 436, but deviates from existing test guidelines for acute toxicity studies, as well as the existing studies examined by RAC, as it includes only one concentration but has a detailed characterisation of the exposure atmosphere and histopathological examination of the airways of exposed rats as well as blood oxygen monitoring.

This study report (redacted of all confidential information) was the subject of a targeted consultation from 23 January to 6 February 2023. In accordance with Article 77(3)(c) of REACH, and in accordance with the Terms of Reference provided to the Committee with the request, RAC has reviewed the available information on acute toxicity by inhalation, taking into account the above-mentioned aspects, and has considered whether to amend the opinion of 5 December 2019 in relation to the classification for acute toxicity by the inhalation route and/or the setting of an ATE for the classification of mixtures. Acute toxicity via the inhalation route is therefore the only hazard class and differentiation considered in this opinion.

RAC general comment

Substance characteristics and read-across of data

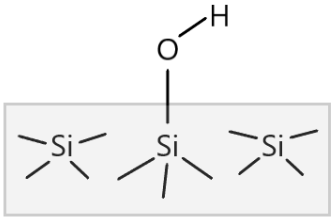
The substance in the scope of the current Annex VI entry is pyrogenic (fumed) silica surface-treated with hexamethyldisilazane. "Silica" is an alternative name for silicon dioxide (SiO_2). Silica can be crystalline (e.g. quartz) or amorphous. Both crystalline and amorphous SiO_2 can be of natural or synthetic origin. Synthetic amorphous silica (SAS) can be prepared by a variety of processes. The so-called wet-route processes occur in liquid phase and yield silica gels, precipitated silicas or colloidal silicas. Fumed (pyrogenic) silicas are produced by flame hydrolysis. In the flame hydrolysis process, $SiCl_4$ is burnt in the presence of oxygen and hydrogen to produce amorphous SiO_2.

Flame hydrolysis produces silica droplets that coalesce and subsequently, on entering the colder area of the flame, partially solidify. The partially solidified droplets (typically 5-20 nm in diameter) collide and merge (sinter) to form larger three-dimensional chain-like aggregates. The typical size of the aggregates is 100-1000 nm (0.1-1 µm). Subsequently, after solidification, the individual aggregates cluster to form agglomerates held together by weak interactions (such as hydrogen bonds and van der Waals forces). The size of the agglomerates may reach hundreds of µm. However, due to the weak bonding, the agglomerates are relatively fragile. They can be split back to aggregates, for instance by shear forces, and then re-agglomerate when the shear forces are no longer present.

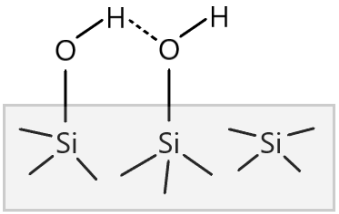
aggregate **agglomerate**

Because of their shape, the aggregates contain a lot of empty space, resulting in a rather low bulk density (or high volume per unit weight) of the material. Tamped density of pyrophoric SAS is typically in the order of 0.05 g/cm^3, compared to its skeleton density (measured by pycnometry) of about 2 g/cm^3. Due to the low density, the geometric particle size is substantially larger than the aerodynamic diameter (defined as the diameter of a sphere of unit density that has the same gravitational settling velocity in air as the particle in question), the latter being a key determinant of deposition in the respiratory tract of particles with an aerodynamic diameter above ≈ 0.5 µm.

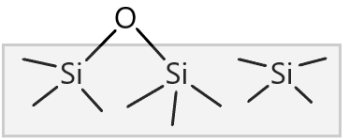
The various types of amorphous silica differ in their surface chemistry. Colloidal silica, precipitated silica and silica gel are highly hydrophilic because of the high silanol group density on their surface. Silicas produced at high temperatures, such as fumed silica, contain silanol groups at a lower density because a large part of silanol groups are dehydrated to siloxane bonds (Si–O–Si) like those forming the body of the particle. As a result, the surface of pyrophoric SAS is less hydrophilic than that of silicas produced by wet processes.



silanol group, isolated

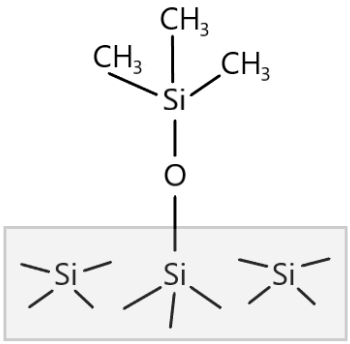


silanol groups, vicinal, bridged with a hydrogen bond

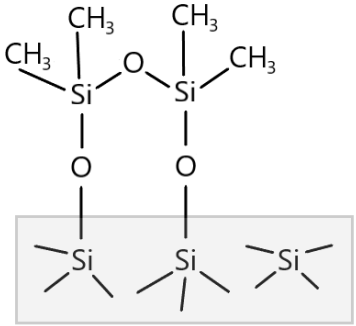


siloxane bond (from dehydrated silanol groups)

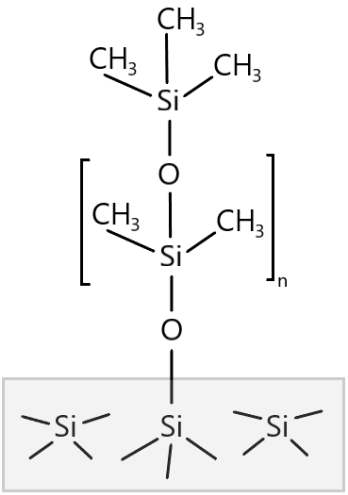
The hydrophobicity of fumed silicas can be further increased by treatment with reagents such as hexamethyldisilazane (HMDZ), dichlorodimethylsilane (DDS) or polydimethylsiloxane (PDMS). The organic functional groups at the surface of HMDZ-, DDS- and PDMS-treated SAS are shown below. A certain portion of the silanol groups remain unreacted.



HMDZ-treated SAS

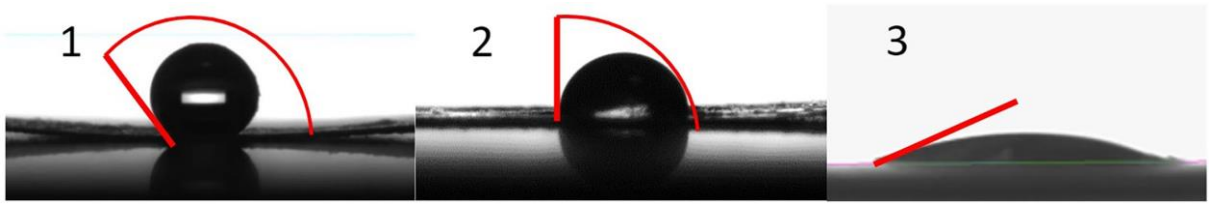


DDS-treated SAS



PDMS-treated SAS

Hydrophobicity can be characterised by determination of contact angle. A drop of water is placed on the test material and the angle between a tangent to the liquid surface and the solid surface at this point is measured. Materials with a contact angle below 90° are generally considered as hydrophilic whereas those with a contact angle above 90° are considered as hydrophobic. Contact angles for (1) HMDZ-treated SAS, (2) untreated pyrogenic silica and (3) untreated silica gel are shown in the photographs below (from Krueger *et al.*, 2022).



Contact angle (from Krueger *et al.*, 2022)**:**

HMDZ-treated SAS **untreated pyrogenic silica** **untreated silica gel**

Given the similarities in surface chemistry between HMDZ-, DDS-, and PDMS-treated SAS, RAC in its opinion in 2019 accepted read-across from DDS- and PDMS-treated SAS to HMDZ-treated SAS for health hazards. This conclusion is still considered valid.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity via the inhalation route

Summary of the basis for the Art. 77(3)c request

Industry, represented by the Association of Synthetic Amorphous Silica Producers (ASASP), submitted a report of a new mechanistic acute inhalation toxicity study with HMDZ-treated SAS (Anonymous, 2022; also published as Krueger *et al.*, 2022; the study is described under 'Assessment and comparison with the classification criteria'). This study reported 100% mortality at the single concentration tested of 0.5 mg/L. Examination of the respiratory tract was claimed to confirm physical obstruction of the nasal cavity as the mode of action (MoA) of lethality. Industry further argued that the suffocation effects observed in rats do not represent intrinsic toxicity, cannot be extrapolated to humans due to anatomical and physiological differences, and should therefore not be used for classification. The argumentation of industry stakeholders is presented in more detail under 'Comments received during targeted consultation' and then discussed in the RAC assessment part.

Comments received during targeted consultation

Comments were received from 8 industry associations, 1 company-importer and 4 individuals.

All commenters were in favour of no classification. The main points of their argumentation can be summarised as follows (please note that the following text represents the commenters' position and interpretation of data):

- The new acute inhalation toxicity study (Anonymous, 2022) combined detailed characterisation of exposure atmosphere (including particle size and stability of the test atmosphere) with histopathological examination of a wide range of tissues including the nasal cavity. To avoid removing any deposited particles, the nasal cavities were shock frozen before histopathological examination.
- The new study demonstrated a complete physical obstruction of nasal cavities by the test material. The observed clinical signs, such as preterminal gasping, and macroscopic findings in the lungs such as congestion, oedema, acute emphysema and petechiae, as well as lung histopathology (haemorrhage, acute necrosis, acute emphysema) are also consistent with asphyxia. The test material was not detected in the lungs by energy dispersive X-ray (EDX) analysis.
- In order to produce a respirable test atmosphere, agglomerates have to be broken down using considerable shear forces. Upon inhalation, they re-agglomerate and deposit in the respiratory tract of animals. Obstruction of airways with deposited test material is not a specific intrinsic property of the substance but a purely physical effect that can be expected as a result of the loading of the airways with any material of similar density, hydrophobicity, and particle size.
- The observed physical obstruction should not be misdiagnosed as a toxic effect, as stated in the OECD guidance document (GD) 39 (2018), paragraph 69: "At very high concentrations, dry powder aerosols […] tend to form conglomerates in the proximal nose causing physical obstruction of the animals' airways (e.g., dust loading) and impaired respiration which may be misdiagnosed as a toxic effect."
- The observed suffocation effect in rats cannot be transferred to humans due to differences in respiratory airways anatomy and breathing patterns. Rats are obligate nasal breathers, whereas humans are both nose and mouth breathers. Thus, blockage of the nasal passages results in suffocation in rats but not in humans. Further, the diameter of the

upper airways is larger and the airflow higher in humans than in rats. If particles inhaled at high concentrations reached the lower airways in humans, the major interspecies differences (e.g. higher alveolar volume or coughing reflex in humans) will also result in different outcomes. Therefore, a fatal outcome from the blocking of upper or lower airways in humans by inhaled hydrophobic SAS is highly unlikely.

- According to the CLP regulation (e.g. Art. 5), "the information used for classification shall relate to the forms or physical states in which the substance is placed on the market and in which it can reasonably expected to be used". HMDZ-treated SAS is commercially used as non-respirable agglomerates with a mass median aerodynamic diameter (MMAD) of approx. 80 µm. The particle size of the material tested in acute inhalation toxicity studies differs significantly from that which can reasonably be expected in the commercial product under normal handling and use conditions.
- In the older acute inhalation toxicity studies (i.e. those conducted prior to Anonymous, 2022) the range of examinations was limited (when compared to the new study) and the test atmospheres were not properly characterised (due to the complexities of MMAD determination for this type of material). Therefore, these studies should be assigned a Klimisch score of 3 (i.e. not reliable).

Assessment and comparison with the classification criteria

Acute inhalation toxicity study in rats with HMDZ-treated SAS (Anonymous, 2022; Krueger et al., 2022)

The design of this GLP study was based on OECD TG 436 but included additional investigations such as histopathological examination. The test material was Aerosil R812 (manufactured by Evonik Operations GmbH). As noted above, only one concentration was tested.

Test atmosphere

The toxicity study was preceded by technical pre-tests without animals (Stintz and Wessely, 2022; Wessely *et al.*, 2022). The particle size in the pre-test was simultaneously measured by cascade impactor and laser diffraction. Laser diffraction was considered a more suitable method for this type of material because the shear forces in the impactor break the fragile agglomerates to smaller particles. As a result, the MMAD determined by the impactor is smaller than the MMAD of the atmosphere in exposure chambers. When interpreting the result of the pre-test, it should be borne in mind that, due to the low density of the material, the geometric particle size from the laser diffraction measurements had to be converted to aerodynamic particle size by dividing by a factor of 4 (square root of 1/0.064, calculated from tamped density of 0.064 g/cm^3).

The pre-tests showed that generation of OECD TG-compliant aerosol atmosphere (i.e. MMAD 1 to 4 µm) over a period of 4 h is possible at a concentration of 600 mg/m^3 (0.6 mg/L). The average MMAD by laser diffraction at this concentration was ca. 4.5 µm, by cascade impactor below 0.5 µm. At 5100 mg/m^3 (5.1 mg/L) the particle size (measured by cascade impactor) increased above the recommended range already after 10 minutes due to agglomeration. Only one MMAD determination was made in the toxicity test itself, with cascade impactor before the start of the exposure, showing a MMAD ± GSD of 0.50 ± 1.31 µm at a concentration of about 500 mg/m^3. The corresponding MMAD by laser diffraction can be estimated at around, or slightly above, 4 µm. No laser diffraction measurement took place in the toxicity test.

Mortality

In the acute toxicity study, 3 male and 3 female Wistar rats (strain Crl:WI (Han)) were exposed nose-only to the test material at a mean concentration of 517 mg/m^3 (0.517 mg/L). The intended exposure duration of 4 hours was shortened to 3 h 12 min due to mortalities. The timepoints of

deaths and associated clinical signs are listed in the table below. Originally it was planned to measure blood oxygen level with pulse oximeter (out of GLP) in all animals after exposure. However, due to mortality and moribundity this was only possible in two animals (1201 and 1203, saturation 75% and 124% respectively approx. 1 hour after the end of exposure).

Anonymous (2022): mortality and clinical signs		
Time after the start of exposure	**Event**	**Clinical signs in the affected animal**
2 h 45 min	1 male (1102) found dead	1102, 5 min before death: reduced respiratory rate
3 h 5 min	1 male (1103) and 1 female (1202) found dead	1103 and 1202, prior to death: reduced respiratory rate, signs of anemia
3 h 12 min	Exposure ended, the remaining 3 animals (1101, 1201, 1203) transferred to their cages	
4 h 10 min	1 male (1101) and 1 female (1201) found dead The remaining 1 female (1203) killed in moribund condition	1101, 1201: respiratory distress 1203: moribund condition, respiratory distress

When 100% mortality is observed at 0.5 mg/L in an OECD 436 study, testing at 0.05 mg/L normally follows. This was not done in Anonymous (2022). Industry clarified that "*for animal welfare reasons, the study was not designed to determine the LC_{50}, which is already known based on the old studies. Therefore, it was unnecessary to test multiple concentrations*" (Dekant, 2023). They also stated in the document that "*The core aim of the new study was to prove the hypothesis of a blockage of the airways*". Nevertheless, RAC is of the view that testing at multiple concentrations, even if the number of animals per concentration was reduced, would provide valuable additional information on the MoA. The use of a single concentration, which caused 100% mortality, could be considered to make the results inconclusive with respect to MoA.

Pathology

All animals were necropsied approx. 10 minutes after death. On gross pathology all animals were found to have dark red lungs, some also foamy contents in the trachea. Lung weights of all animals exceeded historical control mean. The macroscopic changes were further described as congestion, oedema, acute emphysema and petechiae.

Histopathological examination of the lungs revealed alveolar haemorrhage, alveolar fibrin, acute emphysema and alveolar macrophages in most or all animals; in addition, some animals showed alveolar wall necrosis and mixed cell infiltrate. The severity of the histopathological findings was from minimal to slight. Individual lung histopathology data are presented in Appendix 1. No histopathological findings were identified in other respiratory or non-respiratory tissues except thymus haemorrhages, representing petechiae.

Nasal cavities from the three animals that died during exposure (1102, 1103, 1202) were frozen (to avoid loss of the substance by rinsing out with fixing solution), dried, cryo-sectioned, silver coated and examined under digital microscopy for deposition of foreign material. The study report documents almost complete blockage of nasal cavity by deposited test item for animal no. 1202, partial blockage for animal no. 1102 and multisite deposition of test substance in the nasal cavity but not obstruction for animal no. 1103. The test substance was found in a form of large

particulate structures of gel-like appearance. The sections were then examined by scanning electron microscope / energy dispersive X-ray (SEM/EDX) analysis for the presence of the test substance (outside GLP requirements). Large areas of accumulated Si were detected in the nasal cavities of all three animals.

Nasal cavities from the remaining three animals (1101, 1201, 1203) were also frozen and subsequently fixed in 100% ethanol and embedded in methylmethacrylate resin before sawing. Si could not be allocated by EDX in the resin-embedded nasal cavities, probably because Si detection was prevented by the resin (the EDX beam can penetrate only to a limited depth). Light microscopy examination of the resin-embedded nasal tissues is not mentioned in the study report.

As a follow-up of the RAC-65 CLH Working Group discussion, industry was asked to provide information on deposition of test substance animals 1101, 1201 and 1203. The following clarifications were received (in a document dated 16 May 2023): To avoid removal of test substance deposits during the fixation of the tissues, new methods were needed, as the usual ones using formalin may lead to dissolution or removal of the material. As there was no experience with such methods, two different methods – shock freezing and epoxy embedding were selected. In the methyl methacrylate embedded nasal cavities, partial or total blockage was also visible by digital microscopy (photographs provided in the document). Due to fixation process with alcohol, the test item was probably partly washed out or dissolved during the embedding process. Thus, the findings were not as conclusive as in the frozen and dried nasal cavities. Therefore, these observations in the methyl methacrylate embedded nasal cavities were not further discussed in the study report.

RAC took note of this clarification, although it is not entirely clear why a liquid fixative was used at all.

The information on deposition of test substance in nasal cavities available in the study report and in the additional document (May 2023 is summarised in the following table.

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

EDX analysis of the larynx, trachea and lung (from frozen and dried samples) revealed slight amounts of Si in the larynx of animal 1102 and a minimal amount in the trachea of animal 1203.

Histologically, these findings were not supported by any change in larynx, trachea, bronchial bifurcation and carina. No Si was detected by EDX in the lungs.

Parallel studies with other materials

In the document dated 16 May 2023 industry also briefly referred to experiments of the same design using the same concentration of 500 mg/m^3 with six other test items (particles), performed in parallel to Anonymous (2022). Lung histopathology findings at the 24-hour interim sacrifice included increased presence of reactive alveolar histiocytes, macrophage type II proliferation, and mixed inflammatory infiltrate or interstitial inflammation. These findings were noted for 5 out of 7 tested materials and were associated in two studies with the presence of foreign material in the alveoli.

RAC interpretation of the findings with regard to the possible mode of action

No remarkable histopathological changes were observed in the wide range of non-respiratory tissues examined. Given that HMDZ-treated SAS is a poorly soluble particulate material of low toxicity, mortality in an acute inhalation study must be related to effects in the respiratory tract. In principle, such particles can affect the respiratory system by two modes of action: (1) obstruction or (2) inflammation.

Partial or almost complete obstruction of the nasal cavity by the test substance was demonstrated for 5 out of 6 examined animals. No significant amounts of deposits were detected in the larynx, trachea or lungs. Obstruction of nasal cavity in obligate nose breathers like rats can lead to mortality. However, complete or almost complete blockage was demonstrated only for some animals. Therefore, the findings in the nasal cavity have to be interpreted together with lung histopathology.

The pattern of lung histopathology in humans and animals killed by oro-nasal occlusion has been described e.g. by Brinkmann *et al.* (1984). The typical features include focal emphysema, multiple focal intra-alveolar and interstitial haemorrhages, congestion, alveolar macrophages and interstitial oedema.

Typical features of particle-related acute inflammation include increased alveolar macrophages, inflammatory infiltrate (often suppurative), sometimes also acute necrosis, congestion and oedema (Renne *et al.*, 2009).

Histopathological examination in the current study revealed multiple haemorrhages, focal emphysema, alveolar macrophages, and, to a minor extent, mixed cell infiltrate. This pattern of effects is consistent with suffocation. Even though alveolar macrophages and mixed cell infiltrate can be also indicative of inflammation, the observed degree of severity (grade 1, minimal) does not appear sufficient to explain the mortalities.

Thus, the mortalities in the current study were probably due to suffocation caused by obstruction of nasal cavity by deposited test material.

Previous acute inhalation toxicity studies with hydrophobic surface-treated SAS

The CLH report (ANSES, 2018) presented a single acute inhalation toxicity study with DDS-treated SAS (Anonymous, 1983) showing no mortality at the highest attainable concentration of 477 mg/m^3. The CLH report further described a mechanistic study (Anonymous, 2005), where female rats were administered HMDZ-treated SAS in physiological saline via single intratracheal instillation at dose levels up to 1.2 mg/lung. The animals were sacrificed after an observation period of 3, 21 or 90 days. There was no mortality of clinical signs. BAL (bronchoalveolar fluid) analysis after 3 days showed increased number of cells, mainly neutrophils and macrophages,

and increased protein content, which is consistent with an inflammatory reaction. The BAL parameters on days 21 and 90 were similar to vehicle control levels.

A number of additional acute inhalation toxicity studies with hydrophobic SAS were identified in the open literature (ECETOC, 2006; Becker *et al.*, 2013) by RAC in 2019, and most of them showed mortalities. A study with DDS-treated SAS reporting an LC_{50} of 450 mg/m^3 (Anonymous 1994a) was selected by RAC at that time as a basis for the conclusion that the substance should be classified as Acute Tox. 2; H330 (RAC, 2019).

In the present assessment RAC re-examined all available study reports of rat acute inhalation toxicity studies with HMDZ-, DDS-, and PMDS-treated SAS. The list of reviewed studies, together with a description of relevant findings, is provided in Appendix I.

The focus of the present assessment is on the MoA of lethality. Most valuable for this purpose are studies in which histopathological examination of the respiratory tract was performed. Histopathological investigation was conducted in 3 out of the 12 reviewed studies, namely in Anonymous (1981a), Anonymous (2000) and Anonymous (1996c). These studies are discussed in more details below.

Anonymous (1981a)

5 males and 5 female rats were exposed (whole-body) for 4 hours to PDMS-treated SAS (Cab-O-Sil N70TS) at a single concentration of 4900 mg/m^3 (MMAD 0.36 µm, cascade impactor). All animals died during exposure. The lungs of the animals failed to collapse on necropsy. The lungs of all males were subject to histopathological examination (reported in an addendum to the study report: Anonymous, 1982a).

Tabular description of histopathology listed two findings: acute diffuse foreign body bronchitis, bronchiolitis and alveolitis (severe), and multifocal alveolar emphysema (mostly moderate). These two findings were present in all examined animals. The pathology report further contains the following narrative description: "The deaths were the result of a foreign body reaction in the lungs. A pinkish staining foreign substance was present in the larger bronchi and has completely occluded some of the smaller bronchioles. In some areas, this substance extended to alveolar spaces. The foreign material did not contain any specific identifiable material as it was non-birefringent under polarized light. Acute necrosis of the bronchial and bronchiolar epithelium was visible in some areas, and acute haemorrhage was present multifocally at the alveolar level. The alveolar emphysema was the result of the accumulation of the foreign material in the airways." The pathologist interpreted the findings as "blockage and irritation of the respiratory tree" that appeared to be "primarily due to the physical presence of the foreign material rather than a direct toxic effect of the substance."

This study was designed as a limit test with a target concentration of 5 mg/L. It was followed by a second 4-hour study with a target concentration of 2 mg/L (actual 2.2 mg/L; Anonymous 1981b), where all 10 animals died during exposure. A third study (Anonymous 1982b) with Cab-O-Sil N70TS reported 30% mortality at 1.3 mg/L, but it was a 1-hour exposure, so the result is not directly comparable to the previous two studies. Subsequent 4-hour studies with other materials from the same manufacturer (Cabot) indicated LC_{50} values in the order of 0.5 mg/L and 100% mortality starting around 1 mg/L (Anonymous 1994a, Anonymous 1994b, Anonymous 2000).

RAC notes that under the conditions of study Anonymous (1981a), i.e. at a concentration of ca. 5 mg/L, the test material was able to reach the bronchi, bronchioles and alveoli and cause serious lung effects (severe inflammation, moderate emphysema, occlusion of bronchioles by foreign material). However, the regulatory relevance of effects at a concentration approx. 5-fold above the lowest concentration causing 100% mortality is questionable.

Anonymous (2000)

Rats were exposed (nose-only) for four hours to DDS-treated SAS (Cab-O-Sil TS-610) at concentrations 520, 1120 and 2790 mg/m^3. The mortality was 0/10, 14/14 and 10/10 respectively (note the steep dose response). Only the group exposed at 1120 mg/m^3 was subject to histopathological examination. MMAD at 1120 mg/m^3, measured by aerodynamic particle sizer, was around 0.8 µm throughout exposure period. Cascade impactor was not considered a suitable method for this test substance as almost all material ended up at the back filter, implying an MMAD below 0.1 µm. Out of the 7 males and 7 females of this group, 13 animals died during exposure and 1 was killed in extremis after exposure (on the day of exposure). Gross examination showed haemorrhagic lungs of reduced elasticity and white powder in the nasal cavity of all animals.

2 males and 2 females were subject to histopathological examination of the nasal cavity, larynx, trachea and lungs. Tabular description of lung findings listed alveolar erythrocytes (focal, moderate to severe), eosinophilic oedema (little to some, predominantly perivascular), bronchiolar lumina with several nucleated cells and eosinophilic material (in some animals up to occlusion), and slight bronchiolar epithelial erosion. Larynx of three of the four examined animals showed a luminal plug of pale eosinophilic material with few nucleated cells and erythrocytes.

The report further contains the following narrative description: "Several alveoli contained erythrocytes and oedema. These features are indicative of acute serous pneumonia. In addition, the epithelium lining of the bronchi/bronchioli was affected: at some sites the epithelial lining was interrupted (eroded), at other sites it was flattened. Goblet cells were scarce. The lumina of the nasopharynx, larynx and the bronchi/bronchioli (from main bronchi till the terminal bronchioli) contained large quantities of pale-eosinophilic material intermingled with nucleated (epithelial) cells and erythrocytes. Especially in the smaller bronchioli, the material filled the entire lumen."

The particle size in this study was probably lower than in Anonymous (2022): MMAD in Anonymous (2000) was 0.8 µm by aerodynamic particle sizer vs ≈ 4 µm by laser diffraction in the new study, or <0.1 µm by cascade impactor vs 0.5 µm in the new study. The concentration in the group used for histopathology (1120 mg/m^3) was somewhat higher than in Anonymous (2022) but just above the LC_{50}. Under these conditions, the substance caused occlusion of the airways throughout the respiratory tract (from the nasal cavity down to the bronchioles). The histopathology is indicative both of obstruction and inflammation. Acute emphysema was not observed. Despite some involvement of inflammation, occlusion of the airways is likely to be the main cause of the observed mortality in this study.

Anonymous (1996c)

The material tested in this study was HDK SKS 300VI, synthetic amorphous silica surface-treated with vinyl-modified HMDZ (vinyl function in HMDZ was below 1% according to ECETOC, 2006). RAC notes that the vinyl modification may have an impact on the physico-chemical properties of the material (e.g. agglomeration tendency) and that this material is not explicitly covered by the accepted read-across approach. Therefore, the study is only presented as supporting information.

Rats (5 sex/group) were exposed nose-only for 4 hours at concentrations of 400, 700 and 2000 mg/m^3 (MMAD 6.9 µm, cascade impactor). The mortality was 2/10, 10/10 and 10/10 respectively. Gross pathology showed red discolouration of the lungs. Nasal tissues were grossly examined as well, no abnormalities are reported. 5 animals per concentration (2-3 of each sex) were subject to histopathological examination of the lungs. The remaining animals (5/concentration) were used for determination of silica content in lung homogenate by atomic absorption spectroscopy.

The histopathological findings in decedents were similar regardless of exposure group (see the table below) and included alveolar haemorrhage (mostly mild), interstitial oedema (trace to moderate) and inflammation (mostly mild). Interestingly, there was no significant difference

between findings in animals that died during exposure and those who died 1 day post-exposure. Overall, the severity of the lung findings was low (mostly trace to mild) and cannot explain the mortality. Detailed examination of other parts of the respiratory tract using appropriate techniques to avoid wash-out of deposits was obviously not performed. Therefore, the pathology findings in this study are inconclusive with regard to the cause of death.

Silica content in the lungs increased in a dose-dependent manner, from 4.0 mg/lung at 400 mg/m^3 to 12.7 mg/lung at 2000 mg/m^3 (for comparison, silica content in the lungs of control animals, 1 per group, ranged between 0.3 and 1.6 mg/lung). The observed silica content in the lungs is quite significant. The MMAD measured by cascade impactor was approx. 7 µm and the actual MMAD may have been much higher (cf. Stintz and Wessely, 2022), so the pulmonary fraction might be expected to be low. Still, it can be estimated that at least 10% of the inhaled dose (assuming a minute ventilation of 0.2 L/min and exposure duration of 4 hours) was deposited in the lungs in this study. This is comparable to the typical lung deposition fraction for particle sizes between 1 and 4 µm (Asgharian *et al.*, 2003). Obviously, a substantial pulmonary deposition of surface-modified SAS may occur at MMADs significantly above 4 µm.

Another significant observation is that despite its confirmed (by chemical analysis) presence in the lungs, the test substance was not observed in this organ on standard histopathological examination.

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

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

Mode of action of lethality in acute inhalation toxicity studies with hydrophobic surface-treated SAS and its human relevance

Mode of action

HMDZ-treated SAS and the read-across substances (DDS- and PDMS-treated SAS) show no acute oral toxicity and no or only a slight skin and eye irritation potential. No remarkable histopathological changes in non-respiratory tissues were observed in animals that died in the acute inhalation toxicity study by Anonymous (2022). As discussed above, particles of

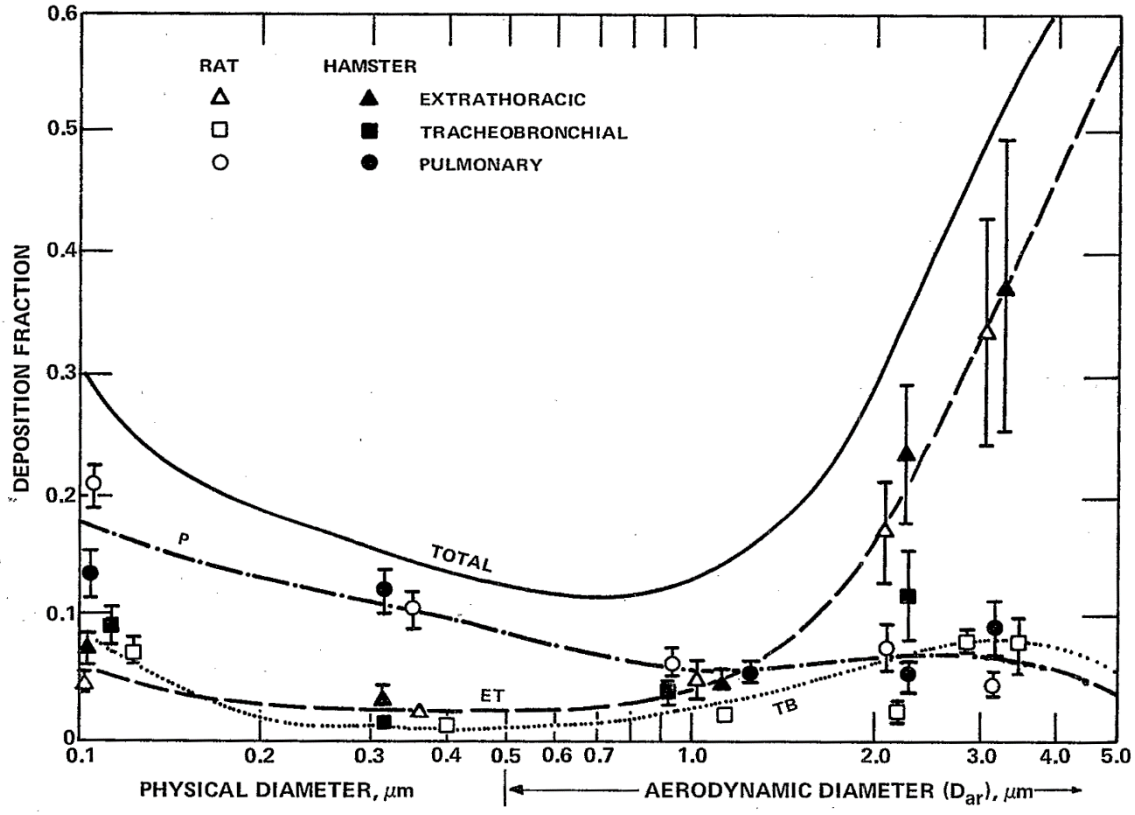
hydrophobic surface-treated SAS can affect respiratory system by two modes of action: (1) obstruction or (2) inflammation.

Lethality in the new mechanistic study (Anonymous, 2022) was probably caused by obstruction of the nasal cavity. On the other hand, the study by Anonymous (2000) reported obstruction of both upper and lower airways together with some lung inflammation. The difference may be partly due to the smaller particle size in Anonymous (2000).

Despite some evidence of inflammation, airway obstruction is likely to have been the main driver of lethality also in Anonymous (2000). Therefore, only occlusion of upper and lower airways will be discussed in the following analysis of human relevance.

Deposition of the substance in the airways of rats and its human relevance

Particle size affects the deposition pattern of the particles in the respiratory tract. According to the OECD TGs, e.g. 436 (2009), the recommended MMAD in acute inhalation toxicity studies is 1 to 4 µm with a geometric standard deviation of 1.5 to 3. Materials of this particle size are likely to deposit within all regions of the respiratory tract of rats (SOT, 1992), as illustrated in the following figure (from US EPA, 1982, p. 11-31):



Deposition of inhaled monodisperse aerosols of fused aluminosilicate spheres in rats and hamsters. ET = extrathoracic region, TB = tracheobronchial region, P = pulmonary region, TOTAL = total respiratory tract (from US EPA, 1982)

Generation of aerosols of this MMAD not only ensures substantial exposure of the alveolar region, but also helps to avoid obstruction of the upper airways of rodents, which could lead to suffocation (SOT, 1992). In contrast to the relatively simple human nasal turbinates, the rodent turbinates show complex folding and branching patterns. As a result, the nose of rodents has a higher filtering efficiency for particles compared to humans, but is also more susceptible to clogging. Further, humans use both nasal and oral breathing, whereas rodents are obligate nose breathers

due to the close apposition of the epiglottis to the soft palate. Thus, obstruction of the nasal cavity may lead to suffocation in rodents but not in humans.

Accordingly, the CLP guidance (Guidance on the application of the CLP criteria, v. 5.0) explains in section 3.1.2.3.2 that "the use of such fine aerosols helps to avoid partial overloading of extra-thoracic airways in obligate nasal breathing species like rats." Similarly, the OECD GD 39 (par. 69) states that "at very high concentrations, dry powder aerosols […] tend to form conglomerates in the proximal nose causing physical obstruction of the animals' airways (e.g., dust loading) and impaired respiration which may be misdiagnosed as a toxic effect." These statements imply that (1) occlusion of the nasal cavity by the test substance in rodent inhalation toxicity studies should be avoided, and that (2) occlusion of the nasal cavity in rodent studies is not expected when the particle size distribution meets the OECD requirements.

Hydrophobic surface-treated fumed silica appears to be an exception in this regard because despite using an OECD-compliant atmosphere (such as in the study by Anonymous, 2022), obstruction of the upper airways by deposited material did occur.

One possible explanation of these unusual properties lies in the low density of the test material (around 0.05 g/cm^3), whose deposition pattern may be different from that of materials with a density in the order of 1 g/cm^3.

Hydrophobicity may be another significant factor. Occlusion of the airways by deposited test substance leading to mortality was reported for some hydrophobic organic pigments. Hofmann *et al.* (2018) tested several organic pigments for acute inhalation toxicity in rats at the limit concentration of 5 mg/L. Out of the seven materials tested, three caused 100% mortality, and four of them caused no mortality at the limit concentration. Histopathological examination in two of the studies showing mortality (azomethine yellow and diketopyrrolopyrrole (DPP) red) revealed marked test substance deposition in various regions of the respiratory tract, including the lungs. Total obstruction of the larynx was observed for DPP red. Lung obstruction was accompanied by diffuse emphysema in the study with azomethine yellow. No mortalities occurred for any material at the next lower concentration of 1 mg/L. The authors tried to find a correlation between mortality and physicochemical properties of the test materials (surface area, surface charge, contact angle). They noticed that the 3 substances causing mortality were the most hydrophobic ones, having a contact angle ca. 140°, compared to 0° to 128° for the 4 substances not causing mortality. For comparison, HMDZ-treated SAS has a contact angle of 147°.

Thus, the tendency of hydrophobic surface-treated fumed silicas to block the upper airways of rodents appears to be related to a combination of hydrophobicity, low solubility and low density of the material.

In line with the CLP guidance and the OECD GD 39, RAC is of the view that **obstruction of the nasal cavity observed in rat studies** with hydrophobic fumed silicas **is not relevant for humans**.

However, the substance also reaches the bronchi, bronchioles and alveoli. As already mentioned, the nose of rats has a higher filtering capacity for particles compared to humans. Consequently, the fraction reaching the post-nasal regions is higher in humans compared to rats (US EPA, 1982, chapter 11.2). Further, humans, unlike rodents, can also breathe via the mouth. The table below illustrates the deposition patterns on nasal and oral breathing in humans (from Heyder *et al.*, 1986). The values are derived from experiments with monodisperse aerosols in three healthy subjects.

Particle deposition patterns in humans at a mean flow rate of 0.25 L/s and a tidal volume of 0.5 L (from Heyder *et al.*, 1986)									
	Nasal breathing (% deposition)					**Oral breathing (% deposition)**			
Particle size (unit density spheres) **(µm)**	**Total**	**Nose**	**La-rynx**	**Bron-chi**	**Alve-oli**	**Total**	**La-rynx**	**Bron-chi**	**Alve-oli**
0.05	33	0	0	0	33	33	0	0	33
0.2	13	0	0	0	13	13	0	0	13
1	26	13	0	0	13	15	0	0	15
2	59	38	2	1	18	28	2	1	25
3	81	56	5	2	18	44	8	4	32
4	92	68	7	3	14	56	16	7	33
10	100	91	7	2	0	86	65	17	4

The lower filtration capacity of the nose and the capability of oral breathing in humans (in contrast to rats) increase the concern about the lung effects in rat studies. On the other hand, the dimensions of airways and alveoli are larger in humans than in rats (e.g. the diameter of a rat alveolus is ca. 100 µm compared to ca. 200 µm in humans), making the lungs of humans somewhat more resistant to overload by deposited material or accumulated alveolar macrophages.

Industry further mentioned differences in tracheobronchial branching pattern (monopodial in rats vs dichotomous in humans), leading to different deposition patterns. However, it is not obvious whether this modifies the level of concern (cf. Hofmann *et al.*, 1996), and if so, in which direction. Similarly, no detailed discussion was provided with regard to interspecies differences in the cough reflex. Nevertheless, it is plausible that at least the material deposited in the area of the larynx can be easily coughed out by humans, unlike rats.

Overall, the interspecies differences are not sufficient to entirely dismiss human relevance of the lung findings in rat acute inhalation toxicity studies with hydrophobic surface-treated SAS. **Obstruction of lower airways by deposited test material is** therefore considered **potentially relevant for humans.**

Relevance of the exposure atmosphere in animal studies to the human situation

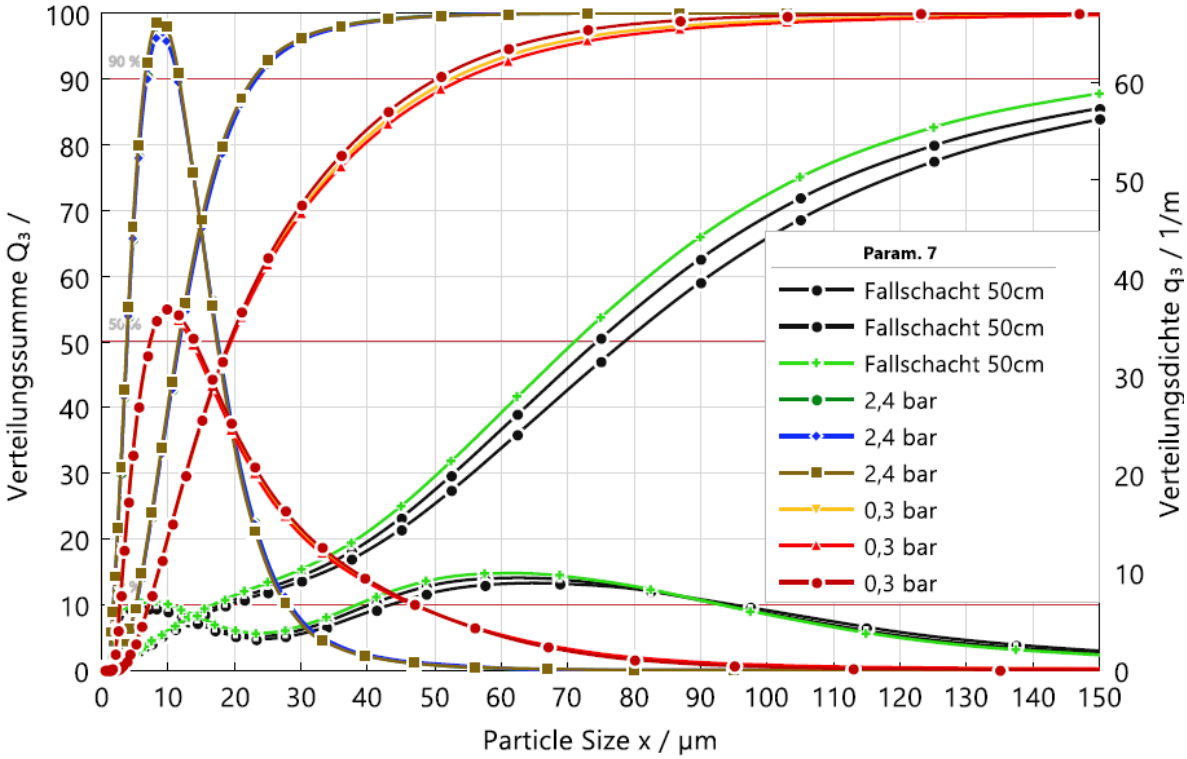
Industry pointed out that according to the CLP regulation (e.g. Article 5), the information used for classification "shall relate to the forms or physical states in which the substance is placed on the market and in which it can reasonably expected to be used." Weber and Dekant (2022) informed that "HMDZ-treated SAS is commercially used as non-respirable agglomerates with a MMAD of approx. 80 µm. Significant shear stress is required to break down the agglomerates into particles in the respirable range (MMAD < 10 µm) for inhalation toxicity testing. The small particles generated by shear stress readily re-agglomerate when shear stress is absent."

The CLP guidance provides some further explanations in this regard. First, in section 1.2.2 it defines the term 'reasonably expected use' in relation to hazard classification. Reasonably expected use includes activities such as production, handling, spraying, but also reasonably foreseeable accidental exposure. One example of reasonably foreseeable accidental exposure in the current case may be spillage of dust leading to a brief, high exposure.

With regard to human health hazards, the CLP guidance (1.2.3.2) recommends that "in general, testing should be performed on the smallest available particle size." On the other hand, the document (in section 1.2.3.2) clarifies that "in some cases, substances or mixtures have to be transformed into specific forms not mirroring 'real-life' exposure in order that an animal test can be performed. As a consequence, the results of such tests may have to be evaluated taking into account any limitations due to the fact that the specific form of the tested substance or mixture does not or not perfectly represent that to which human exposure may occur during intended, known, or reasonably expected use. Such evaluation has to be performed according to the state of the scientific and technical knowledge."

Similarly, in the chapter dealing with acute toxicity, the CLP guidance (3.1.2.3.2) acknowledges that although "the use of highly respirable dusts and mists is ideal to fully investigate the potential inhalation hazard of the substance", "these exposures may not necessarily reflect realistic conditions. For instance, solid materials are often micronized to a highly respirable form for testing, but in practice exposures will be to a dust of much lower respirability. […] In such situations, specific problems may arise with respect to classification and labelling, as these substances are tested in a form (i.e. specific particle size distribution) that is different from all the forms in which these substances are placed on the market and in which they can reasonably expected to be used."

Weber and Dekant (2022) did not specify which product and measurement conditions they referred to in their statement that the typical MMAD of marketed HMDZ-treated SAS is 80 µm. Stintz and Wessely (2022) presented results of particle size measurements for Aerosil R812 (using laser diffraction) under various shear stress intensities. The results are presented in the graph below.



Aerosil R812 particle size distribution in air under different shear force intensities, measured by laser diffraction (from Stintz and Wessely, 2022)

Under low dispersion intensity (freefall shaft 50 cm; the black and light-green lines), the median geometric particle size was ca. 70-80 µm. This corresponds to an aerodynamic diameter of ca. 20 µm (using a conversion factor of 4). Under high dispersion intensity (injector, dispersion

pressure 2.4 bar; the brown and blue lines) the median geometric particle size decreased to 12 µm (corresponding to an MMAD of approx. 3 µm).

RAC notes that the study by Anonymous (1996c) on pyrogenic silica surface-treated with vinyl-modified HMDZ demonstrated substantial lung exposure in rodents at a MMAD, measured by cascade impactor (i.e. high shear intensity conditions) of 7 µm.

Another factor that has to be taken into account is that the current Annex VI entry covers all HMDZ-treated pyrogenic SAS products, not only Aerosil R812. One of the determinants of the particle size distribution of the agglomerated product is the particle size distribution of the aggregates. Particle size distribution can be modified by varying the conditions of the manufacturing process. It is possible that some of the HMDZ-treated SAS products currently on the market have a smaller median particle size than Aerosil R812, or that products with smaller stable particle sizes will be produced in the future.

Therefore, the argument that the form of hydrophobic surface-treated SAS tested in the acute inhalation toxicity studies is not relevant for human exposure situation is not considered valid.

Intrinsic properties

Industry argued that obstruction of airways with deposited test material is not a specific intrinsic property of the substance but a purely physical effect that can be expected for any material of similar density, hydrophobicity and particle size.

The available information indeed indicates that the tendency of HMDZ-treated SAS to cause airway obstruction and lethality in rats is primarily related to its physico-chemical properties, namely a combination of hydrophobicity, low solubility and low density. RAC assumes that a similar effect can be expected for any substance possessing this combination of physico-chemical properties.

According to the CLP guidance (1.1.3), intrinsic hazards are "the basic properties of a substance or mixture as determined in standard tests or by other means designed to identify hazards." RAC acknowledges that the mortality in acute inhalation toxicity studies with hydrophobic surface-treated SAS is related to the physico-chemical properties of undissolved particles rather than to toxic properties of the dissolved form.

RAC is of the view that the definition of "intrinsic hazard" in the CLP guidance does not exclude the use of physical effects observed in standard studies for classification if similar harmful effects can be expected in humans.

Conclusion on classification

The lowest lethal concentrations in the available acute inhalation toxicity dataset of hydrophobic surface-treated pyrophoric silicas correspond to Category 2 (0.05 mg/L < ATE ≤ 0.5 mg/L). RAC notes that the Anonymous (2022) study indicates an MoA via suffocation. Still, there remains some doubt as to whether this was also the main MoA in the Anonymous (1994a) study, which was used as the basis for the Acute Tox. 2 classification in the previous RAC opinion. Further, RAC notes that the Anonymous (2022) did not provide any dose-response information as only a single concentration was tested.

Anonymous (2000) was also considered relevant with regard to the MoA. In this case, both a non-relevant MoA (obstruction of the nose and larynx) and relevant MoAs (obstruction of bronchi and bronchioles, some inflammation) were probably involved. The LC_{50} was in the range for Acute Tox. 3 (0.5 mg/L < ATE ≤ 1.0 mg/L), but the MMAD was lower than in the Anonymous (2022) study.

In summary, suffocation by nasal obstruction was considered the main cause of death in the Anonymous (2022) study. However, considering also the findings of the older studies at higher concentrations and different MMAD in a weight of evidence assessment, RAC agreed on **no classification for acute toxicity due to inconclusive data**.

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ANNEXES:

Annex 1 Records of the targeted consultation following receipt of new information on the actute inhalation toxicity of Silanamine, 1,1,1-trimethyl-N- (trimethylsilyl), hydrolysis products with silica

Appendix I

Anonymous (2022): individual lung histopathology data

Individual lung histopathology data from the acute inhalation toxicity study by Anonymous (2022) are presented in the following table.

Anonymous (2022): lung histopathology						
Animal no.	1101	1102	1103	1201	1202	1203
Time and mode of death	FD 1 h after exposure	FD during exposure	FD during exposure	FD 1 h after exposure	FD during exposure	SM 1 h after exposure
Left lobe						
Congestion	P	P		P		
Alveolar haemorrhage	1	2		1	1	
Fibrin, alveolar	1	1		1	1	
Alveolar macrophages		1	1	1		1
Infiltrate, mixed	1					
Emphysema, acute		1	1			1
Alveolar wall necrosis						
Right cranial lobe						
Congestion	P					
Alveolar haemorrhage	1					
Fibrin, alveolar	1	1				
Alveolar macrophages			1			
Infiltrate, mixed	1					
Emphysema, acute	1					
Alveolar wall necrosis						
Right middle lobe						
Congestion	P	P				
Alveolar haemorrhage		1			1	
Fibrin, alveolar	1		1	1		
Alveolar macrophages	1		1			
Infiltrate, mixed	1			1		
Emphysema, acute	2	1	1	1	1	1
Alveolar wall necrosis				1		
Right caudal lobe						
Congestion	P	P			P	
Alveolar haemorrhage	1	2			1	1

Fibrin, alveolar	2	1		1	1	1
Alveolar macrophages			1		1	
Infiltrate, mixed			1			
Emphysema, acute		1		1	1	1
Alveolar wall necrosis		1		1		
Accessory lobe						
Congestion	P					
Alveolar haemorrhage						1
Fibrin, alveolar		1				
Alveolar macrophages		1				
Infiltrate, mixed	1					1
Emphysema, acute					2	1
Alveolar wall necrosis						

FD = found dead; SM = sacrificed moribund; P = present; Severity: 1 = minimal, 2 = mild

Effects in previous rat acute toxicity studies with hydrophobic surface-treated SAS

For the purpose of the present evaluation, RAC requested full study reports to all studies with HMDZ-, DDS- and PDMS-treated SAS listed in ECETOC (2006), Table 30. RAC also requested a study report to study Anonymous/Cabot (2000) referred to in Becker *et al.* (2013). All requested study reports were provided to RAC except Dow Corning (1972) and three studies owned by Wacker (study reports no. 712-004, 712-005 and 712-006; referred to as Wacker 1996f,d,b respectively in ECETOC, 2006). The owner of the latter three studies informed RAC that these reports have never been finalized and remained in a draft status due to methodological deficiencies (e.g. inappropriate particle generation).

The table below summarizes relevant information from these study reports with focus on respiration-related clinical signs, gross pathology and, where available, also histopathology. Unless stated otherwise, each study used 5 animals per sex and concentration and the post-exposure observation period lasted 14 days, at the end of which the necropsy of survivors took place. The MMAD was determined by cascade impactor in most of the studies (except Anonymous, 2000), which is not considered an accurate method for this type of material (i.e. a highly dispersible material of low density).

Reference; Study sponsor; Product name; Surface treatment agent; Exposure duration; MMAD; Exposure mode	**Concentration (mg/m^3) and mortality**	**Time of death, clinical signs (related to respiration), gross pathology, histopathology, other relevant information**
Anon. 1983 Degussa Aerosil R974 DDS 4 h 3.4 µm Whole-body	477: 0/10	Clinical signs: none related to respiration Gross pathology: no abnormalities 477 mg/m^3 was the maximum attainable concentration
Anon. 1984 Degussa Aerosil R809 HMDZ 4 h 1.6 µm Whole-body	1094: 0/10	Clinical signs after exposure: slight respiratory distress Gross pathology: no abnormalities
	2863: 0/10	Clinical signs after exposure: severe respiratory distress Gross pathology: no abnormalities
	3730: 10/10	All animals died within 1 day after the exposure Gross pathology: lungs spotted appearance and red discolouration
	5382: 10/10	All animals died within 1 day after the exposure Gross pathology: lungs spotted appearance and red discolouration
	The animals were not observable during exposure due to the density of the mist	
Anon. 1981a; Anon. 1982a (pathology report) Cabot Cab-O-Sil N70TS PDMS 4 h 0.36 µm Whole-body	4900: 10/10	All animals died during exposure Clinical signs: irregular breathing Gross pathology: red depressions on the lungs (10/10), failure of the lungs to collapse (10/10) Histopathology (only males were examined): Acute foreign body bronchitis, bronchiolitis and alveolitis, severe (incidence 5/5). Multifocal alveolar emphysema (mild 1/5, moderate 4/5). Narrative description: "The deaths were the result of a foreign body reaction in the lungs. A pinkish staining foreign substance was present in the larger bronchi and has completely occluded some of the smaller bronchioles. In some areas, this substance extended to alveolar spaces. The foreign material did not contain any specific identifiable material as it was non-birefringent under polarized light. Acute necrosis of the bronchial and bronchiolar epithelium was visible in some areas, and acute haemorrhage was present multifocally at the alveolar level. The alveolar emphysema was the result of the accumulation of the foreign material in the airways." Control group was included: no mortalities, no gross lesions Some exposed animals not visible during portions of the exposure period
Anon. 1981b Cabot Cab-O-Sil N70TS PDMS	2190: 10/10	All animals died during exposure Clinical signs: irregular breathing Gross pathology: red depressions on the lungs, failure of the lungs to collapse

4 h 0.54 µm Whole-body		Control group was included: no mortalities, no gross lesions in the respiratory tract
Anon. 1982b Cabot Cab-O-Sil N70TS PDMS 1 h 0.62 µm Whole-body	1260: 3/10	3 animals died on post-exposure day 1 Clinical signs: irregular breathing (in all animals, in 5 animals persisted for several days) Gross pathology of animals that died: red depressions on the lungs (3/3), failure of the lungs to collapse (3/3) Gross pathology of survivors: red depressions on the lungs (2/7)
	2830: 10/10	7 animals during or shortly after exposure, 3 animals died on post-exposure day 1 Clinical signs: irregular breathing (during and after exposure) Gross pathology: red depressions on the lungs (8/10), failure of the lungs to collapse (8/10), white powder substance in nasal passages (3/10)
	6280: 10/10	All animals died during or shortly after exposure Clinical signs: irregular breathing Gross pathology: red depressions on the lungs (8/10), failure of the lungs to collapse (9/10)
	Control group was included: no mortalities, gross pathology of the lungs showed red depressions (9/30) Some animals at 6280 and 2830 mg/m^3 were not visible during portions of the exposure period	
Anon. 1982c Cabot Aerosil R972 DDS 1 h 0.15 µm Whole-body	2280: 0/10	Clinical signs: irregular breathing (all animals), persisted until post-exposure day 6 to 14 Gross pathology: red discoloration of the lungs 1/10 Control group was included: no mortalities, no gross lesions in the respiratory tract
Anon. 1994a Cabot Cab-O-Sil TS610 DDS 4 h 1.2 µm Whole-body	210: 0/10	Clinical signs during exposure: laboured breathing Gross pathology: lungs with white and red areas (10/10) Post-treatment observation period in this group was 22 days
	540: 7/10	7 animals died during exposure Clinical signs during exposure: laboured breathing, respiratory distress Clinical signs after exposure: dyspnoea (up to post-exposure day 3) Gross pathology of animals that died: lungs larger than normal with red areas (7/7), white material in the nasal turbinates (7/7) Gross pathology of survivors: lungs with white and red areas (3/3)
	2100: 10/10	All animals died during exposure Clinical signs: laboured breathing, respiratory distress Gross pathology: lungs larger than normal with red areas (10/10), white material in the nasal turbinates (10/10)
	LC_{50} 450 mg/m^3; this study was used for setting the ATE in RAC (2019)	

Anon. 1994b Cabot Cab-O-Sil TS530 HMDZ 4 h 1.6 µm Whole-body	90: 1/10	1 animal died on post-exposure day 3. Gross pathology: lungs darker than normal with red areas Gross pathology of survivors: no abnormalities
	840: 8/10	3 animals died during or shortly after exposure, 3 on post-exposure day 1, 2 on post-exposure day 2 Clinical signs after exposure: dyspnoea, tremor Gross pathology of animals that died: dark lungs with red areas (8/8), white material in nasal turbinates (6/8) Gross pathology of survivors: no remarkable findings
Anon. 2000 Cabot Cab-O-Sil TS610 DDS 4 h 0.8 µm Nose-only	520: 0/10	Clinical signs during exposure: decreased and irregular breathing (slight) Clinical signs after exposure: increased breathing rate (until post-exposure day 3) Gross pathology: lungs filled with foam (10/10), white stains on the lungs (10/10)
	1120: 14/14	7 males and 7 females. 2 males and 2 females subject to histopathological examination of the nose, larynx, trachea and lungs 13 animals died during exposure, 1 animal killed moribund after exposure Clinical signs: decreased and irregular breathing Gross pathology: lungs haemorrhagic and reduced elasticity (14/14), white powder in the nasal cavity (14/14) Histopathology: Nasal cavity nasopharyngeal luminal slight pale eosinophilic material, erythrocytes, few nucleated cells (2/4). Larynx luminal plug of pale eosinophilic material, few nucleated cells, erythrocytes (3/4); epiglottal epithelial erosion (1/4). Lungs intra-alveolar erythrocytes (4/4, slight to severe), eosinophilic oedema (3/4, predominantly perivascular); bronchiolar lumina with several nucleated cells, little eosinophilic material (4/4, in 2 animals focal occlusion); slight bronchiolar epithelial erosion, alternating with areas of flattened epithelium (3/4). Narrative description: "Several alveoli contained erythrocytes and oedema. These features are indicative of acute serous pneumonia. In addition, the epithelium lining of the bronchi/bronchioli was affected: at some sites the epithelial lining was interrupted (eroded), at other sites it was flattened. Goblet cells were scarce. The lumina of the nasopharynx, larynx and the bronchi/bronchioli (from main bronchi till the terminal bronchioli) contained large quantities of pale-eosinophilic material intermingled with nucleated (epithelial) cells and erythrocytes. Especially in the smaller bronchioli, the material filled the entire lumen."
	2790: 10/10	All animals died during exposure Clinical signs: shallow breathing, restlessness Gross pathology: lungs petechiae (10/10), nose blocking lumps of white particles and slime (10/10), nasopharynx haemorrhagic (10/10)
	Particle size measured with aerodynamic particle sizer	
Anon. 1996a Wacker HDK SKS130 HMDZ 4 h 7.5 µm	900: 0/10	Clinical signs after exposure: laboured or rapid breathing, in most animals persisted until post-exposure day 1 Gross pathology: no abnormalities
	2200: 4/10	3 animals died on the day of exposure (after the end of exposure), 1 animal died on post-exposure day 1 Clinical signs after exposure: laboured or rapid breathing, in survivors persisted until post-exposure day 1 to 2

Nose-only		Gross pathology of animals that died: severe red discoloration of the lungs Gross pathology of survivors: no abnormalities
Anon. 1996b Wacker HDK SKS300 HMDZ 4 h 7.1 µm Nose-only	400: 3/10	3 animals died on post-exposure day 1 Clinical signs after exposure: rapid respiration Gross pathology of animals that died: severe red discoloration of the lungs Gross pathology of survivors: no abnormalities
	600: 6/10	3 animals died on the day of exposure (shortly after exposure), 3 animals died on post-exposure day 1 Clinical signs after exposure: rapid or laboured breathing (in survivors did not persist beyond post-exposure day 1); in 1 animal gasping and convulsions before death on the day of exposure Gross pathology of animals that died: red discoloration of the lungs Gross pathology of survivors: no abnormalities
Anon. 1996c Wacker HDK SKS300 VI Vinyl-modified HMDZ 4 h 6.9 µm Nose-only	400: 2/10	2 animals died on post-exposure day 1 Clinical signs after exposure: laboured or rapid breathing, in survivors persisted until post-exposure day 2 Gross pathology of animals that died: severe red discoloration of the lungs Gross pathology and histopathology of survivors: no abnormalities Histopathology of the lungs of animals that died: see 2000 mg/m^3 Mean content of the test material in the lungs (survivors): 4.0 mg/lung; control animal: 1.6 mg/lung
	700: 10/10	2 animals died on the day of exposure (1 during exposure), 6 on post-exposure day 1, 2 on post-exposure day 2 Clinical signs after exposure: laboured breathing Gross pathology: severe red discoloration of the lungs Histopathology of the lungs: see 2000 mg/m^3 Mean content of the test material in the lungs: 8.8 mg/lung; control animal: 0.3 mg/lung
	2000: 10/10	8 animals died on the day of exposure (6 during exposure), 2 animals died on post-exposure day 1 Clinical signs after exposure: laboured breathing Gross pathology: severe red discoloration of the lungs Histopathology of the lungs (there were no significant differences between groups, therefore presented for decedents of all groups): congestion (12/12, mostly mild), alveolar haemorrhage (12/12, mostly mild), interstitial oedema (12/12, mostly trace to mild), acute vasculitis (9/12, trace to mild), acute alveolar interstitial inflammation (4/12, trace to mild) Mean content of the test material in the lungs: 12.7 mg/lung; control animal: 1.0 mg/lung
	Histopathological examination of the lungs in 5 animals per concentration (2-3 of each sex) Lung tissue analysis for the test substance (determination of silica by atomic absorption spectroscopy) from 5 animals per concentration (from the animals not used for histopathological examination)	