Expert Review: **Review of acute inhalation studies on SAS and surface-modified SAS to generate an expert statement on classification and labeling needs**

Reviewer: Wolfgang Dekant, Dr. rer. nat.; Professor of Toxicology (em)

[redacted]

e-mail: [redacted]

Dr. Axel Bosch, Fachchemiker für Toxikologie, [redacted]

e-mail: [redacted]

Sponsor: **SASFORREACH Consortium GbR**

**c/o Dr. Knoell Consult GmbH**

Marie-Curie-Straße 8

51377 Leverkusen

Germany

[redacted]

Feb. 17, 2020

W. Dekant, Professor of Toxicology — Date

[redacted]

Feb. 17, 2020

A. Bosch, PhD — Date

# Table of contents

Executive summary ..... 2

1. Introduction ..... 3

2. Results of the acute inhalation toxicity testing with surface-modified SAS ..... 4

3. Criteria for classification regarding toxicity after single inhalation exposures ..... 8

4. Rationale for non-classification of surface-treated SAS for acute toxicity after inhalation or STOT-SE ..... 9

4.1 No classification for acute toxicity warranted ..... 9

4.1.1 Lethal effects of SAS in the inhalation studies are due to suffocation by particle-induced blockade of the airways ..... 9

4.1.2 Particle size distribution of commercial SAS is different from the particle sizes used in the inhalation toxicity testing ..... 10

4.1.3 The lower $LC_{50}$-values reported for surface-modified SAS are likely due to differences in the kinetics of agglomeration ..... 11

4.1.4 Human relevance of suffocation in rodents after high particle loads ..... 12

4.1.5 Chemical composition and reactivity of SAS ..... 12

4.2 No classification for STOT-SE warranted ..... 12

4.2.1 Absence of intrinsic toxicity of surface-modified SAS ..... 12

4.2.2 Experimental results on inhalation toxicity of SAS were generated with a particle size different from that of the commercial material ..... 13

5. Conclusions ..... 13

6. References ..... 14

## Executive summary

Acute inhalation studies with some types of surface-modified (hydrophobic) synthetic amorphous silicas (SAS) indicated high mortality resulting in four hour $LC_{50}$ values that ranged from 80 to > 2,000 mg/m$^3$. Formally, some of the $LC_{50}$ values with surface-modified SAS are in the range of guidance values for classification regarding acute inhalation toxicity under GHS.

However, lethality of surface-modified SAS under toxicity testing conditions is due to airway obstruction by the large particle load received. This results in suffocation. Thus, lethality is not due to a specific property of SAS. $LC_{50}$ values obtained with non-surface modified (hydrophilic) SAS were generally higher as compared to surface-modified SAS (ECETOC, 2006). The differences in potency between SAS to cause lethality can be explained by differences in agglomerization kinetics.

Suffocation as a cause of lethality in rodents after inhalation of SAS under toxicity testing conditions has no relevance to humans exposed to SAS placed on the market. The particle size distribution of the SAS used in the inhalation toxicity testing is significantly reduced to fulfill 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. This aspect needs to be considered in hazard definition of surface-modified SAS. SAS placed on the market only contain a very small mass percentage of respirable particles that may reach the deeper respiratory tract and specifically the terminal bronchioli that are susceptible to obstruction. Lethality due to suffocation therefore should not be used as basis for classification. Moreover, as CLP requires classification of materials based on information that "shall relate to the forms and physical states in which the substance is placed on the market and in which it can reasonably expected to be used", the observations made in the acute inhalation toxicity studies with much smaller particles as compared to those used commercially have no/very limited relevance for classification.

## 1. Introduction

Both non surface-modified and surface-modified synthetic amorphous silica (SAS) have many uses. SAS is intentionally manufactured and does not contain detectable levels of crystalline silica. SAS is produced either by a thermal process (high temperature hydrolysis of chlorosilanes – called pyrogenic or fumed silica) or by a wet process (precipitation of a water glass solution with acids – called precipitated silica, colloidal silica and silica gel). In the first step of the production process of SAS, primary particles with dimensions below 100 nm are formed. These primary particles convalently bind to form aggregates (indivisible units), which have no physical boundaries among them. The aggregates have external dimensions typically above 100 nm (pyrogenic, precipitated, gel). These aggregates further combine to form agglomerates in the micron size range by physical attraction forces (van der Waals) and hydrogen-bridges (aSASp, 2018c). SAS powder is placed on the market as micron-sized agglomerates with an internal structure in the nanoscale.

Obtained SAS can be modified by surface treatment to generate a hydrophobic character. In such surface-modified SAS, surface silanol groups are modified by a chemical reaction with a functional silane or a siloxane and up to 70 % of the silanol groups on the surface of a SAS particle may be modified. All commercial SAS products, besides colloidal silica that produced as a dispersion of particles mostly in water, are white and fluffy powders or dispersions of these powders.

Under realistic conditions, dry SAS is present in the form of agglomerated aggregates with much larger particle sizes (> 100 μm MMAD, mass median aerodynamic diameter) as

compared to those that are required in the OECD-guidelines (MMAD < 4 μm) for inhalation toxicity testing. The large particles present in commercial SAS are not respirable and significant shear stress is required to break down SAS agglomerates to respirable particles as required in the testing guideline. Small SAS agglomerates and aggregates formed by the application of shear stress have a high tendency to re-agglomerate, especially at higher concentrations. These characteristics of surface-modified SAS permit to propose a plausible mode of action for lethality after exposures to very high concentrations of respirable SAS-particles.

Formally, the low $LC_{50}$ values of some surface-modified SAS in some studies may require classification regarding acute inhalation toxicity. In addition, the classification criteria according to regulation (EC) 1272/2008 (on classification, labeling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (CLP)) as specific target organ toxicant (STOT) – single exposure, inhalation (mist/dust) under the Globally Harmonized System of Classification and Labeling of Chemicals (GHS, 2013) may be met since reversible or irreversible adverse health effects were observed at a relative low particle masses in air.

As a classification for health effects has to rely on expert opinion, this statement evaluated the available database on the acute toxicity of surface-modified SAS and the physicochemical characteristics of surface-modified SAS to derive an evidence-based conclusion regarding a need for classification of SAS for acute inhalation toxicity as required in the appropriate guidance in the CLP-regulation.

## 2. Results of the acute inhalation toxicity testing with surface-modified SAS

An overview of the available studies is presented in table 1.

Acute inhalation studies with some types of surface-modified SAS indicated high mortality (most animals died during or shortly after the end of the inhalation exposures) with an apparent lethal concentrations expressed as four hour $LC_{50}$ of 80 $mg/m^3$ (ECETOC, 2006) indicating a need for classification for acute toxicity. In addition, the study results could be interpreted that non surface-modified SAS may have higher $LC_{50}$ values as compared to surface-modified SAS.

However, the toxicity database is complex due to the many different SAS types marketed, differences in study design, problems to obtain stable test atmospheres due to sedimentation of suspended SAS aggregates and agglomerates, rapid agglomeration of SAS in the absence of shear stress in the exposure chambers, and the generally limited parameters assessed in single exposure inhalation studies. While most studies followed respective guidelines for acute inhalation toxicity testing and report to have generated particle sizes (expressed as mass median aerodynamic diameter, MMAD) in the ranges required for

inhalation toxicity testing of particulate material (MMAD < 4 μm), the reported MMADs have to be considered questionable due to complexities of correctly determining particle sizes of SAS (Stintz, 2001; Stintz *et al.*, 2008). In some cases, MMADs of the particles actually reaching the breathing zone of the test animals were not determined. This is important due to the high tendency of SAS to re-agglomeration, specifically at high particle concentrations (see below). Yields of respirable particles in the toxicity studies with SAS were usually low (comparison nominal *vs.* actual concentration) and most studies needed to introduce very large amounts of SAS into the exposure systems to obtain the desired actual concentrations of respirable particles in the size range required by the testing guideline. Some studies reported that animals could not be observed during the exposures due to the extreme dust concentrations in the exposure chambers. Due to the many issues described, any conclusions on the potential of the surface-modified SAS listed in table 1 for acute toxicity is severely hampered by the questionable validity of the reported MMADs and thus the highly uncertain $LC_{50}$-values.

**Table 1:** Overview of acute inhalation toxicity studies with different surface-modified SAS (from ECETOC, 2006, modified) based on an evaluation of the original study reports

| Type/ Product name | Strain, number, sex (per group) | Surface treatment agent | Particle size MMAD (µm) | Exposure duration (h) | Intended concentration (mg/m$^3$) | Nominal concentration (mg/m$^3$) | Health Effects Clinical observations/necropsy | Mortality (substance groups) | $LC_{50}$ (mg/m$^3$) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Pyrogenic SAS | | | | | | | | | | |
| CAB-O-SIL® N70TS fumed silica | SD 5 M, 5 F | Polydimethyl-siloxane CAS 67762-90-7 | 0.54 | 4 | 0 (control), 2,190 (whole body) | 13,900 | Irregular breathing, absence of visible life signs/depression of the lung, red; failure of the lungs to collapse | 10/10 (shortly after exposure) | < 2,190 | Cabot, 1981e |
| CAB-O-SIL ® N70TS fumed silica | SD 5 M, 5 F | Polydimethyl-siloxane | 0.62 | 1 | 0 (control), 1,260, 2,830, 6,280 (whole body) | 13,200 41,900 93,200 | Irregular breathing, absence of visible life signs, crusty nose and eyes, squinting, poor coat quality, some animals not visible during exposure/depression of the lung, red; failure of the lungs to collapse, opacity eyes, white powder in nasal passages | 3/10 10/10 10/10 (shortly after exposure) | Probably between 1,260 and 2,830 | Cabot 1982b,c |
| CAB-O-SIL ® TS 610 fumed silica | Wistar 5 M, 5 F ; lowest concentration 7 M, 7 F | Dichloro-dimethylsilane | 0.7 | 4 | 2,790 1,120 520 (nose only) | 4,000 1,690 560 | Irregular, shallow, decreased breathing; restlessness/petechiae on the lungs, blocking lymps by white particles, slime in nose, hemorrhagic nasopharynx, and lungs, white powder in the nasal cavity, soiled fur; oxygen uptake severely impaired, larger bronchioles, bronchi and nasopharynx partly occluded with cell debris and test material | 10/10 10/10 | between 520 and 1,690 | Cabot, 2000 |
| CAB-O-SIL ® TS 530 fumed silica | Wistar 5 M, 5F | Hexamethyl-disilazane | 0.95, 2.15 | 4 | 90 840 (whole body) | | Red staining of nose, mouth; eyes closed, lacrimation, fur coated with test substance, piloerection, dyspnoe, tremors, wetness urogenital area, lethargy, soling urogenital area, diarrhea, ptosis, chromodacryorrhea/wet anogenitial area, opaque eyes, white material in nasal tubinates, dark lungs with red areas, | 1/10 8/10 | between 90 and 840 | Cabot, 1994b |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | white material in nasal tubinates, larger dark lungs with red areas, intestines with read areas | | | |
| HDK® SKS 130 | SD 5 M, 5 F | Hexamethyl-disiloxane | 0.2 | 4 | 350<br>770<br>2,530<br>5,300<br>(whole body) | 910<br>1,900<br>6,000<br>15,000 | Rapid respiration, decreased activity, labored breathing/severe read discoloration of lungs | 0/10<br>0/10<br>10/10<br>10/10 | Probably between 770 and 2,530 | Wacker, 1996a |
| HDK® SKS 130 | SD 5 M, 5 F | Hexamethyl-disiloxane | 7.2<br>7.7 | 4 | 900<br>2200<br>(nose only) | 13,100<br>25,800 | Labored breathing, rapid respiration, decreased activity, increased salivation/red discoloration of lungs | 0/10<br>4/10 | > 2200 | Wacker, 1996b |
| HDK® SKS 300 | SD 5 M, 5 F | Hexamethyl-disiloxane | 7.1<br>7.0 | 4 | 400<br>600<br>(nose only) | 2,900<br>4,800 | Rapid respiration, decreased activity, labored breathing, loss of righting reflex, convulsions | 3/10<br>6/10 | estimated between 400 and 600 | Wacker, 1996c |
| HDK® SKS 300 VI | SD 5 M, 5 F | Hexamethyl-disiloxane with vinyl function (< 1%) | 0.1 | 4 | 80<br>340<br>1,200<br>5,000<br>(whole body) | 300<br>1,200<br>4,700<br>21,000 | labored and rapid respiration, decreased activity/severe red discoloration of lungs | 5/10<br>10/10<br>10/10<br>10/10 | 80 | Wacker, 1996d |
| HDK® SKS 300 VI | SD 5 M, 5 F | Hexamethyl-disiloxane with vinyl function (< 1%) | 6.3<br>7.7<br>6.6 | 4 | 2,000<br>700<br>400<br>(nose only) | 9,700<br>4,100<br>2,000 | labored and rapid respiration, decreased activity, excessive lacrimation/severe red discoloration of lungs, granular extracellular material in alveoli (presumed to be test material) | 10/10<br>10/10<br>2/10 | estimated between 400 and 700 | Wacker, 1996e |
| Aerosil® R 972 | Not specified 5 M, 5 F | Dichloro-dimethylsilane | 0.15 | 1 | 2,280 | 13,700 | Irregular breathing, crusted eyes and nose, crusted muzzle, yellow/brown stained fur, squinting, alopecia/no abnormalities in 6/10 animals, depression red lungs, discoloration | 0/10 | > 2,280 | Cabot, 1982 |
| Aerosil R 974 | Wistar 5 M, 5 F | Dichloro-dimethylsilane | 56% < 5; calculated MMAD: 2.9 * | 4 | 477 (technically highest achievable concentration)** | 24,400 | Restlessness, closed eyes/no abnormalities | 0/10 | > 477 | Degussa, 1983 |

* Density of approx. 0.05 g/cm3; ** Explanation for the high nominal concentrations is that Aerosil R 974 mainly consists of large agglomerates, which have great settling speeds under the influence of gravidity. Also seen with Aerosil R 972 (Klosterkötter, W., Archiv für Hygiene und Bakteriologie 149 (1965), Heft 7/8).

## 3. Criteria for classification regarding toxicity after single inhalation exposures

The CLP-criteria for classifying chemicals under acute inhalation toxicity and STOT-SE are summarized in tables 2 and 3. Classification for acute inhalation toxicity is typically based on potency of the mist/dust to induce adverse effects after four-hour inhalation exposure tests in animals such as OECD 403 (OECD, 2009b) and OECD 436 (OECD, 2009a). Guidance values for categorization of the potency inhalation of mists/dust have been developed regarding effects after single inhalation exposures (table 2). The simple endpoint lethality is used as a measure of an adverse effect applying the estimated $LC_{50}$ (in simple mass units such as $mg/m^3$ in air) to distinguish between different categories.

**Table 2**: Guidance values to assist in classification for acute inhalation toxicity for dust exposures (CLP, Table 3.1.1 forth row re-arranged)

| Category | Hazard Statement | $LC_{50}$ value (mg/m3) |
|---|---|---|
| 1 | Fatal if inhaled | ≤ 50 |
| 2 | Fatal if inhaled | > 50 and ≤ 500 |
| 3 | Toxic if inhaled | > 500 and ≤ 1000 |
| 4 | Harmful if inhaled | > 1000 and ≤ 5000 |
| 5 | May be harmful if inhaled | This category includes substances which are of relatively low acute toxicity hazard but which under certain circumstances may present a danger to vulnerable populations. |

The corresponding text to the above table in the CLP regulation states:

"The acute toxicity estimate (ATE) for the classification of a substance …. is derived using:

— the $LD_{50}/LC_{50}$ where available,

— the appropriate conversion value from Table 3.1.2 (in the respective version of the CLP-regulation) that relates to the results of a range test, or

— the appropriate conversion value from Table 3.1.2 (in the respective version of the CLP-regulation) that relates to a classification category"

The STOT-SE criterion applies to significant, non-lethal toxic effects observed after single exposures and may cover effects such test-item related significant changes in organ structure and function, adverse changes in clinical biochemistry, respiratory irritation, etc. Allocation of a test item to categories 1 and 2 (in table 3 below) are typically based on adverse effects observed during four-hour inhalation exposure tests in rats and in the

required observation period after the end of the inhalation (14 days), whereas Category 3 is typically based on human data.

**Table 3:** Guidance values to assist in STOT-SE classifications for dust exposures (GHS, Chapter 3.8 and CLP regulation)

| Category | Hazard Statement | Concentration (single-dose exposure causing significant non-lethal effects) |
|---|---|---|
| 1 | Causes damage to organs | ≤1 000 mg/m$^3$ |
| 2 | May cause damage to organs | > 1 000 to ≤ 5 000 mg/m$^3$ |
| 3 | May cause respiratory irritation; or<br>May cause drowsiness or dizziness | Guidance values are not provided since this classification is primarily based on human data |

## 4. Rationale for non-classification of surface-treated SAS for acute toxicity after inhalation or STOT-SE

As stated in the CLP-guidance, classification of a chemical substance shall be determined on the basis of the total weight of evidence using expert judgment. Moreover, in the context of SAS particles, the following aspects in the CLP regulation concerning relevance of data for hazard classification have to be considered:

"*The information shall relate to the forms or physical states in which the substance is placed on the market and in which it can be reasonable be expected to be used*"

"*Tests that are carried out for the purpose of this regulation shall be carried out on the substance or on the mixture in the form(s) of physical state(s) in which the substance or mixture is placed on the market and in which it can be reasonably be expected to be used.*"

Based on an analysis of the study reports on inhalation toxicity of SAS and considering of the issues of inhalation testing for acute toxicity of materials with complex physicochemical properties such as surface-modified SAS, the following facts argue against a classification of surface-modified SAS for acute toxicity.

### 4.1 No classification for acute toxicity warranted

#### *4.1.1 Lethal effects of SAS in the inhalation studies are due to suffocation by particle-induced blockade of the airways*

The surface-modified SAS used in the testing were reported to exhibit in most cases a particle size distribution with almost 100 % of the present particles with MMADs < 4 μm. As discussed above, very large amounts of particles have to be introduced into the exposure systems to reach high concentrations of small agglomerates or single aggregates of SAS. As these SAS particles are respirable and reach the terminal bronchioli, they are deposited

there in large amounts due to rapid re-agglomeration in the absence of shear stress. Moreover, the humid surfaces of the lung further promote re-agglomeration of surface-modified SAS. Deposition of a large particle mass is confirmed in the more detailed evaluations of pathological changes in single exposure inhalation studies in rats (table 1 and ECETOC, 2006). These confirm that large amounts of inhaled SAS particles were deposited in the lower airways and often completely occluded both bronchi and smaller bronchioles. This frequently observed particle deposition induces the observed lethality in the studies due to mechanical obstruction of airways causing a severe impairment of oxygen uptake into blood resulting in suffocation and death. Since an examination of particle deposition is not required in the testing guideline, it is therefore not frequently assessed. However, additional effects seen in the exposed animals in many of the acute toxicity tests (high lethality during particle exposures, steep dose response for lethality, difficulty breathing, full recovery of the surviving animals within a short time frame) are additional indications of suffocation as cause of death.

Thus, the adverse effects of SAS after inhalation of high concentrations of respirable particles are due to the physical presence of large masses of SAS in the lower airways (ECETOC, 2006). Such effects can also occur after single dose inhalation of large masses of other small lipophilic particles (Hofmann *et al.*, 2018). Therefore, the observed lethality is not due to an intrinsic (toxic) property of SAS. Absence of an intrinsic toxic property of SAS is also indicated by the very low potential for acute toxicity of SAS when applied dermally or orally. $LD_{50}$-values for SAS after both oral and dermal single dose application are well above 2 000 mg/kg bw. Classification of chemical substances for toxicity is to be made on intrinsic toxic properties. Therefore, the effects seen in the acute toxicity studies should not be used as a basis for classification regarding toxic effects.

#### *4.1.2 Particle size distribution of commercial SAS is different from the particle sizes used in the inhalation toxicity testing*

According to CLP regulation, tests performed for the purpose of the regulation shall be carried out on the substance in the form of physical state in which the material is placed on the market and in which it can “be reasonably expected to be used”. The relevant particle size distribution for both non surface-modified and non surface-modified SAS present in commercial marketed products are the sizes of their agglomerates with mean agglomerate sizes in the order of several hundred microns (Stintz, 2001) and the weight fraction of agglomerates/aggregates that may reach the thoracic and alveolar section (MMAD < 4 μm) represents only < 1 % of the overall size-distribution of airborne particles (according to EN/DIN 481) (Stintz, 2001). This size-distribution also applies to normal handling and use conditions (Stintz, 2001).

Continuous application of high shear stress is necessary to obtain a significant reduction of the agglomerate sizes of SAS to obtain a high proportion of respirable particles with an MMAD below 4 μm as required by the guidelines for acute inhalation toxicity testing. Therefore, the available tests do not represent the toxicological behavior of the commercial surface-modified SAS products as such (ECETOC, 2006) due to different particle size distribution of the mechanically modified SAS used in the inhalation toxicity. The testing guideline requiring the presence of respirable particles for hazard characterization and the CLP-regulation requesting testing of particulate materials as "they are placed on the market" are at odds. As a consequence, the information obtained in the hazard assessment applying the required OECD-guidelines to testing of SAS does not permit conclusions on the toxicological behavior of the commercial SAS products (ECETOC, 2006) due to the large differences in particle size distribution of the SAS used in the inhalation toxicity as compared to the marketed SAS. As CLP (see above) requires classification of material on properties of the form it is "placed on the market and in which it can be reasonably be expected to be used", the results of the acute inhalation toxicity studies have no/very limited relevance for classification of commercial SAS.

### *4.1.3 The lower $LC_{50}$-values reported for surface-modified SAS are likely due to differences in the kinetics of agglomeration*

Under realistic conditions, dry surface-modified SAS as placed on the market is present in the form of larger agglomerates that are not respirable. Significant shear stress is required to generate inhalable smaller agglomerates or single aggregates in a highly dynamic equilibrium. In acute inhalation toxicity studies, surface-modified SAS generally show lower nominal $LC_{50}$ values as compared to non surface-modified SAS. These differences are due to differences between in agglomeration kinetics due to a reduction of the agglomeration forces (van der Waals interactions and hydrogen bridges) as a result of a more hydrophobic surface. Formation of larger and non-respirable agglomerates is therefore slower for surface-modified SAS. For small agglomerates or single aggregates of non surface-modified SAS, agglomeration to non-respirable particles is very rapid under the low shear stress environment as present in the breathing zone of animals in the exposure chamber. These differences in physicochemical properties influence the time course of the concentration of respirable particles and correspondingly the effective dose of particles to the lung. For surface-modified SAS with lower agglomeration forces, inhalable particles will be present in a dust atmosphere for a longer time resulting in higher doses of small agglomerates/single aggregates to the terminal bronchioles. This results in airway obstruction by surface-modified SAS at lower nominal concentrations as compared to non surface-modified SAS. Nevertheless, rapid re-agglomeration and sedimentation of the generated inhalable particles

in air at concentrations relevant for acute inhalation testing are observed for both non surface-modified and surface-modified SAS.

#### *4.1.4 Human relevance of suffocation in rodents after high particle loads*

As the high lethality of some surface-modified SAS is due to particle accumulation in the lower airways resulting in a blockade of the airways due to a physical barrier, species differences in airway anatomy need to be included in assessment of human relevance of the reported rodent data. Terminal airways of the rat have a much smaller diameter as compared to terminal bronchioles in humans and are thus much more prone to clogging by precipitated particles and the resulting suffocation. Therefore, in this case, the rodent model is less suitable to define human hazard. Therefore, the reported $LC_{50}$-values have little human relevance.

#### *4.1.5 Chemical composition and reactivity of SAS*

Both non surface-modified and surface-modified SAS contain silicon and oxygen connected in a three-dimensional macromolecule network. Pyrogenic silica typically contains at least 99.8 % SiO2 and precipitated silica, colloidal silica and silica gel typically contain at least 95 % SiO2. The hydrophilic nature of non surface-modified SAS is due to the presence of silanol groups on the surface. Surface-modified SAS considered in this paper usually are obtained by a chemical reaction with hexamethyldisilazane, dimethyldichlorosilane, or polydimethylsiloxanes. The first compound forms mono-functional moieties upon hydrolysis, whereas the latter two give rise to bi-functional units (ECETOC, 2006; Canada, 2013). In the most commercially available surface-modified SAS, the modifying agents are no longer detectable. Surface-modified SAS contain two entities on their surface: (1) short chain polydimethylsiloxane units, which project off the surface of the silica and somewhat shield the remaining hydrophilic entities, and (2) unmodified surface silanols (US-EPA, 2003).

### 4.2 No classification for STOT-SE warranted

#### *4.2.1 Absence of intrinsic toxicity of surface-modified SAS*

Effects seen in the acute inhalation studies can be explained by mechanical airway obstruction due to the high particle deposition in the lung. Neither systemic effects on other parameters nor effects on the lungs of surviving animals that are indicative of a lung specific toxicity were observed in animals sacrificed after the end of the recovery period. The reported remaining changes in the lungs are the consequences of the high particle deposition in the inhalation exposures and thus do not represent an adverse effect of surface-modified SAS and indicate an intrinsic toxicity of hydrophobic SAS. Therefore, again due to arguments summarized under 4.1.1, the observations should not serve as a basis for classification with STOT-SE.

### *4.2.2 Experimental results on inhalation toxicity of SAS were generated with a particle size different from that of the commercial material*

As discussed under 4.1.1, the studies indicating lethality were generated with a specifically generated particle size of SAS that do not represent the commercially available materials. Therefore, as argued above, the results should not be used as basis for classification.

## 5. Conclusions

The database on the acute inhalation toxicity of surface-modified SAS is complex and all study results need to be interpreted with caution due to issues with particle generation, particle size measurements and stability of respirable particles in the test atmospheres due to the need to continuously apply high shear stress. All studies needed to apply very high particle loads. The very high load of particles that readily form (larger) agglomerates when deposited in the lung thus resulted in effects (obstruction of the airways with lethality due to suffocation) that are not an intrinsic property of the hydrophobic SAS, but a result of the extreme load of the rodent lung with particulate material. Besides suffocation due to airway obstruction, toxic effects to other organs besides the respiratory tract after SAS-inhalation were not seen in the acute toxicity studies and did also not occur in repeated dose studies with SAS after oral administration. Moreover, the acute inhalation toxicity studies with surface-modified SAS intentionally use a form of test material with a much higher respirable fraction than is present in commercial surface-modified SAS. Therefore, the acute inhalation studies do not characterize an intrinsic toxicological effect of the commercial surface-modified SAS products. As a consequence, classification of SAS for Acute Inhalation Toxicity and STOT-SE is not warranted. The higher potential of surface-treated SAS for acute lethality is most likely due to the slower re-agglomeration of the generated respirable particles in the exposure chamber to large agglomerates due to reduced binding forces as compared to non surface-modified SAS. This slower re-agglomeration results in larger particle doses to the lung since inhalable particles will be present for a longer time in the chamber atmosphere for non surface-modified SAS as compared to surface-modified SAS.

An acute inhalation toxicity study with a SAS-type with a low $LC_{50}$ that integrates a detailed pathological assessment of all exposed animals including determination of particle deposition in the airways and of oxygen or oxyhemoglobin concentrations in blood during the exposure or in animals that died during the exposures and shortly afterwards may further strengthen this conclusion.

## 6. References


aSASp, 2012. Association of Synthetic Amorphous Silica Producers: Factsheet Synthetic Amorphous Silica (SAS). available online at: https://www.asasp.eu/images/Publications/Nano_-_SAS_factsheet_-_201209.pdf.

aSASp, 2013. Association of Synthetic Amorphous Silica Producers: Statement for Synthetic Amorphous Silica regarding the definition of 'nanomaterials' for cosmetic use in the European Union by the Association of Synthetic Amorphous Silica Producers (ASASP), an Industry Sector Group of Cefic.

aSASp, 2016a. Association of Synthetic Amorphous Silica Producers: Study demonstrates worker safety at Synthetic Amorphous Silica (SAS) Production Plants.

aSASp, 2016b. Association of Synthetic Amorphous Silica Producers: Synthetic Amorphous Silica (SAS) industry response to the opinion of the Scientific Committee on Consumer Safety (SCCS).

aSASp, 2017. Association of Synthetic Amorphous Silica Producers: Statement for Synthetic Amorphous Silica regarding the definition of "engineered nanomaterials" for use in food in the European Union.

aSASp, 2018a. Association of Synthetic Amorphous Silica Producers: Statement regarding the EFSA opinion on the re-evaluation of silicon dioxide (E 551) as a food additive.

aSASp, 2018b. Association of Synthetic Amorphous Silica Producers: Statement regarding the Status of Synthetic Amorphous Silica under French Decree 2012-232 and the Ministerial Order on the Declaration of "Substances at Nanoscale".

aSASp, 2018c. Association of Synthetic Amorphous Silica Producers: Synthetic Amorphous Silica – ASASP's current interpretation as a nanomaterial.

aSASp, 2019a. Association of Synthetic Amorphous Silica Producers: Comments on the Proposal for Harmonised Classification & Labelling (CLH) of Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica as STOT RE 2 – H373.

aSASp, 2019b. Association of Synthetic Amorphous Silica Producers:Synthetic Amorphous Silica (SAS) industry response to the opinion of the Scientific Committee on Consumer Safety (SCCS) on silica in cosmetics with regard to solubility.

BAuA, 2017. BAuA Federal Institute for Occupational Safety and Health Report F 2336: Methodology for the identification of granular biopersistant particles (GBP) at workplace (www.baua.de/dok/8708694).

BPC, 2019. Opinion of the Biocidal Products Committee (BPC) for classification of synthetic amorphous silicon dioxide, surface treated with hexamethyldisilazane/HMDZ (CAS #68909-20-6, silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica) STOT - RE 2 H373 - comments on this proposed classification.

Cabot, 1972a. Hazleton Laboratories Study Report 178-125: Acute inhalation exposure in rats. Federal Hazardous Substances Labeling Act. Test material CAB-O-SIL EH-5.

Cabot, 1972b. Hazleton Laboratories Study Report 178-126: Acute inhalation exposure in rats. Federal Hazardous Substances Labeling Act. Test material CAB-O-SIL M-5.

Cabot. 1982. One hour acute dust inhalation toxicity study in rats of R-972 Degussa surface treated fumed silica. Unpublished report 420-0897 by Toxigenics, Decatur, Illinois, USA. Cabot, Tuscola, Illinois, USA.

Cabot, 1981a. Toxigenics' study 420-0689: Four-hour acute dust inhalation study in rats of CAB-O-SIL N70TS (= TS-720).

Cabot, 1981b. Toxigenics' study 420-0777: Four-hour acute dust inhalation study in rats of CAB-O-SIL N70TS.

Cabot, 1981c. Toxigenics' study: 420-0690: Four-hour acute dust inhalation toxicity study in rats of CAB-O-SI fumed silica M-5.

Cabot, 1982a. Toxigenics' study 420-0826: One hour acute dust inhalation study in rats of CAB-O-SIL N70TS (= TS-720).

Cabot, 1982b. Toxigenics' study 420-0897: One hour acute dust inhalation study in rats of R-972 Degussa surface treated fumed silica.

Cabot, 1982c. Toxigenics' study 420-1103: Acute inhalation study in rats of CAB-O-SIL N70TS. Addendum summary of histopathologic observations of lung tissue for Toxigenetics' study 420-0689.

Cabot, 1994a. MB Research Laboratories' study MB 94-3664 E: Inhalation toxicity study in rats, test article TS-530.

Cabot, 1994b. MB Research Laboratories' study MB 94-3665 E: Inhalation toxicity study in rats, test article TS-610.

Cabot, 2000. TNO-report V99.1036: Acute (4-hour) inhalation toxicity study with TS-610 in rats.

Canada, E., 2013. Environment Canada. Screening Assessment for Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, reaction products with ammonia, octamethylcyclotetrasiloxane and silica. Availalable at http://www.ec.gc.ca/ese-ees

Degussa. 1983. Acute inhalation toxicity study of Aerosil R974 in rats. Unpublished report V 83.112/221216 by Appelman LM, CIVO Institutes TNO, Zeist Netherlands. Degussa, Hanau, Germany.

Degussa, 1983a. Civo Institues TNO study 83.111/221216: Acute inhalation toxicity study of Sipernat 22S in rats.

Degussa, 1983b. Civo Institues TNO study 83.142/221216: Acute inhalation toxicity study of Aerosil 200 in rats.

Dekant, W., 2011. Expert review for Wacker: Synthetic aorphous silica (SAS) - Evaluation of inhalative toxicity of surface-treated (hydrophobic) as compared to untreated (hydrophilic) SAS

Dekant, W. and Colnot, T., 2012. Expert review for SAS for REACH: Synthetic aorphous silica (SAS) - Hazard Evaluation after oral or inhalation exposure.

Dekant, W. and Colnot, T., 2013. Expert review for SAS for REACH: Synthetic aorphous silica (SAS) - Are the results of toxicity studies comparable both within and between SAS forms (pyrogenic, precipitated, gel, colloidal).

ECETOC, 2006. European Centre for Ecotoxicology and Toxicology of Chemicals. Joint Assessment of Commodity Chemicals (JACC) Report N° 51: Synthetic Amorphous Silica. ECETOC, Brussels, Belgium.

ECHA, 201X. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures.

Fruijtier-Polloth, C., 2012. The toxicological mode of action and the safety of synthetic amorphous silica-a nanostructured material. Toxicology **294**, 61-79.

GHS, 2013. United Nations: Globally Harmonized System of Classification and Labeling of Chemicals (GHS) rev. 5.

Grace, 1971. TRW/Hazleton study 161-211: Acute inhalation toxicity - LC50 - rats with silica gel (Syloid 378).

Hofmann, T., Ma-Hock, L., Teubner, W., Athas, J.-C., Neubauer, N., Wohlleben, W., Veith, U., End, N., Gröters, S., van Ravenzwaay, B. and Landsiedel, R., 2018. Reduction of Acute Inhalation Toxicity Testing in Rats: The Contact Angle of Organic Pigments Predicts Their Suffocation Potential. Applied In Vitro Toxicology **4**, 220-228.

Landsiedel, R., Ma-Hock, L., Wiench, K., Wohlleben, W. and Sauer, U.G., 2017. Safety assessment of nanomaterials using an advanced decision-making framework, the DF4nanoGrouping. J Nanopart Res **19**, 171.

OECD, 2009a. Organization for Economic Co-operation and Development – Environment Directorate. Guidance Document on Acute Inhalation Toxicity – Acute Toxic Class Method. Test No. 436. Available at: https://www.oecd.org/env/test-no-436-acute-inhalation-toxicity-acute-toxic-class-method-9789264076037-en.htm

OECD, 2009b. Organization for Economic Co-operation and Development – Environment Directorate. Guidance Document on Acute Inhalation Toxicity. Test No. 403. Available at: https://www.oecd.org/env/test-no-403-acute-inhalation-toxicity-9789264070608-en.htm.

Pauluhn, J., 2014. Derivation of occupational exposure levels (OELs) of low-toxicity isometric biopersistent particles: How can the kinetic lung overload paradigm be used for improved inhalation toxicity study design and OEL-derivation? Part Fibre Toxicol **11**, 72.

Riediker, M., Zink, D., Kreyling, W., Oberdorster, G., Elder, A., Graham, U., Lynch, I., Duschl, A., Ichihara, G., Ichihara, S., Kobayashi, T., Hisanaga, N., Umezawa, M., Cheng, T.J., Handy, R., Gulumian, M., Tinkle, S. and Cassee, F., 2019. Particle toxicology and health - where are we? Part Fibre Toxicol **16**, 19.

Stintz, M., 2001. Technical report: Particle analysis of pyrogenic (fumed) silicas at technical concentrations and under technical handling conditions.

Stintz, M., Barthel, H., Heinemann, M. and Weis, J., 2008. Particle Size Distribution of Fumed Silica Agglomerates at Low Shear Stress, Organosilicon Chemistry, from Molecules to Materials. WILEY-VCH Verlag GmbH & Co. KGaA, pp.

Wacker. 1996a. Acute inhalation toxicity evaluation on SKS 130 in rats. Unpublished report 712-006 by [redacted], MPI Research, Mattawan, MI. Wacker Silicones Corporation, Adrian, Michigan, USA.

Wacker. 1996b. Acute inhalation toxicity evaluation on SKS 130 in rats. Unpublished report 712-009. [redacted], MPI Research, Mattawan, MI. Wacker Silicones Corporation, Adrian, Michigan, USA.

Wacker. 1996c. Acute inhalation toxicity evaluation on SKS 300 in rats. Unpublished report 712-008. [redacted], MPI Research, Mattawan, MI. Wacker Silicones Corporation, Adrian, Michigan, USA.

Wacker. 1996d. Acute inhalation toxicity evaluation on SKS 300 VI in rats. Unpublished report 712-004. [redacted], MPI Research, Mattawan, MI. Wacker Silicones Corporation, Adrian, Michigan, USA.

Wacker. 1996e. Acute inhalation toxicity evaluation on SKS 300 VI in rats. Unpublished report 712-010 by [redacted], MPI Research, Wattawan, MI. Wacker Silicones Corporation, Adrian, Michigan, USA.

Weber, K., Bosch, A., Bühler, M., Gopinath, C., Hardisty, J.F., Krueger, N., McConnell, E.E. and Oberdörster, G., 2018. Aerosols of synthetic amorphous silica do not induce fibrosis in lungs after inhalation: Pathology working group review of histopathological specimens from a subchronic 13-week inhalation toxicity study in rats. Toxicology Research and Application **2**, 2397847318805273.

US-EPA, 2003. US Environmental Protection Agency. Revised test plan for dichlorodimethylsilane reaction products with silica. High Production Volume (HPV) Challenge.