SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Silicon dioxide; synthetic amorphous silicon dioxide (nano)

EC No 231-545-4

CAS No 7631-86-9

Evaluating Member State(s): The Netherlands

Dated: 9 July 2021

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Evaluating Member State Competent Authority

The Netherlands

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Year of evaluation in CoRAP: 2012

Before concluding the substance evaluation a Decision to request further information was issued on: 11 March 2015.

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

Synthetic amorphous silica (SAS) was originally selected for substance evaluation in order to clarify concerns about:

- Other hazard based concern
- Other exposure/risk based concern

During the evaluation no other concern was identified.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

SAS is approved for use in biocidal products for product-type 18 with an end date of 31/10/2025. This applies to the synthetic amorphous silica gel obtained by wet-process, which includes both “precipitated silica” and “silica gel” (EC No 231-545-4, CAS RN 112926-00-8). The other types and forms of SAS are not covered by the biocidal product assessment.

SAS (EC No 231-545-4, CAS RN 7631-86-9) is approved under Regulation (EC) No 1107/2009 as repellent in plant protection products under the condition that a maximum of 0,1 % of particles of Crystalline Silica have a diameter below 50 µm.

Under the regulation for FCMs (Food Contact Materials) and articles made of plastics and recycled plastics there is a restriction for synthetic amorphous silicon dioxide (EC No 231-545-4, CAS RN 7631-86-9) concerning primary particles of 1-100 nm which are aggregated to a size of 0,1 - 1 µm which may form agglomerates within the size distribution of 0,3 µm to the mm size.

SAS is registered as a nanomaterial under REACH, in the EU cosmetics inventory and in the Belgian nano inventory.

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in the table below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION	
Conclusions	**Tick box**
Need for follow-up regulatory action at EU level	X
Harmonised Classification and Labelling	X
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	X
No need for regulatory follow-up action at EU level	

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

4.1.1. Harmonised Classification and Labelling

At present there is no harmonised classification for SAS.

The concern investigated was repeated dose toxicity via the inhalation route of exposure. The concern was founded on the outcome of various repeated dose inhalation studies. The new 90-day inhalation study (Anonymous, 2020), as generated upon the request in the substance evaluation decision, provides additional information on repeated dose inhalation toxicity, including insight in the effects induced, the influence of surface area on toxicity, and (ir)reversibility of the effects.

Adverse effects were observed in the nose, lungs and lymph nodes in particular after exposure to the low surface area form (SAS 2 in the study).

The adverse effects induced by the high surface form (SAS 1) were more limited in incidence, less severe and mostly reversible.

Also noteworthy is the recent evaluation of a closely related substance Silanamine (1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica (EC No 272-697-1, CAS RN 68909-20-6)) by the ECHA's Committee for Risk Assessment (RAC) in December 2019 (ECHA, 2019). RAC concluded that a classification as, amongst others, STOT RE Cat 2, H373 (lungs, inhalation) is justified. The effects induced by silanamine are very similar to those induced by SAS, including inflammation of the lung tissue, fibrogenesis and possibly fibrosis.

Based on the adverse effects observed the evaluating Member State Competent Authority (eMSCA) concludes that there is sufficient ground to draft a proposal for harmonised classification and labelling (CLH) for the endpoint repeated dose toxicity via inhalation.

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

Not applicable.

4.1.3. Restriction

Not applicable.

4.1.4. Other EU-wide regulatory risk management measures

Considering the high potency of in particular SAS 2 and the new insights obtained on the link between substance characteristics and toxicity, a RMOA should be considered after the classification process has been finalised. In this RMOA it should be evaluated whether any additional actions, such as the derivation of an Occupational Exposure Limit (OEL), are necessary to ensure the safe use of SAS.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

Not applicable.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS

Indication of a tentative plan is not a formal commitment by the evaluating Member State. A commitment to prepare a REACH Annex XV dossier (SVHC, restrictions) and/or CLP Annex VI dossier should be made via the Registry of Intentions.

Table 3

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
Proposal for a harmonised classification according to Article 37(1) of the CLP Regulation.	To be determined	The Netherlands
RMOA	After the CLH process	To be determined

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

SAS was originally selected for substance evaluation in order to clarify concerns about:

- Other hazard based concern
- Other exposure/risk based concern

During the evaluation no other concern was identified.

Table 4

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome / conclusion**
Physicochemical properties of each individual SAS form (excluding surface treated forms)	Decision annulled by a Board of Appeal decision (BoA, 2017)
Inhalation repeated dose toxicity with four pyrogenic non-surface treated SAS forms	Inhalation toxicity was investigated for two forms with different surface areas and was confirmed, harmonised C&L process to be initiated.
Physicochemical properties of each individual surface treated SAS form	Decision annulled by a Board of Appeal decision (BoA, 2017)
Toxicity of surface treated SAS	Decision annulled by a Board of Appeal decision (BoA, 2017)

7.2. Procedure

Silicon dioxide was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2012 by the Competent Authority of the Netherlands. SAS comprises the following four types[2]: pyrogenic SAS, precipitated SAS, silica gel and colloidal SAS. The initial grounds for concern were related to the substance characterisation, nanoparticles and toxicity of different forms of the substance.

The eMSCA conducted a targeted evaluation that does not include a full evaluation of all elements of the registration dossier. The evaluation is targeted to the characterisation of the substance, human health hazard assessment in relation to subchronic effects via the inhalation route and exposure assessment of the registered synthetic amorphous silica. During the evaluation, there have been several meetings with the registrants in which the substance evaluation was discussed.

In December 2014 the Member State Committee (MSC) reached agreement on the draft decision and the decision was adopted in March 2015. Requested in the original Decision were:

1. Information on the following physicochemical properties of each individual SAS form […] that is manufactured, imported and/or placed on the market:

   (a) The granulometry, which shall include primary particle size, aggregate/ agglomerate size, and particle size distribution (number-based). […];

[2] SAS types: pyrogenic silica, precipitated silica, silica gel and colloidal silica. SAS forms: all individual size grades and trade names that can be identified separately per SAS type, based on differences in characteristics

(b) The specific surface area (by volume). […];

(c) The hydroxylation state. […];

(d) The water solubility. […];

(e) The density. […];

(f) The dustiness. […];

(g) The point of zero charge. […].

2. Sub-chronic toxicity study (90-day; OECD 413), in rats via the inhalation route with four pyrogenic SAS forms

3. Information on the uses of each individual form of SAS […] that is manufactured, imported and/or placed on the market

4. Information on the following physicochemical properties of each individual surface-treated SAS form […] that is manufactured, imported and/or placed on the market:

(a) The granulometry, which shall include primary particle size, aggregate/ agglomerate size, and particle size distribution (number-based). […];

(b) The specific surface area (by volume). […];

(c) The hydroxylation state. […];

(d) The water solubility. […];

(e) The density. […];

(f) The dustiness. […];

(g) The point of zero charge. […].

5. All toxicological information on surface-treated SAS as manufactured, imported and/or placed on the market as available to the Registrant(s)

In June 2015, the registrants logged an appeal in which it was requested to annul the entire Decision (cases A-014-2015 and A-015-2015). In November 2016 the hearing of the board of appeal (BoA) took place. The BoA concluded in June 2017 that all requests except the second (the 90-day inhalation study) were to be annulled (BoA, 2017).

The sub-chronic inhalation toxicity study had to be performed by the deadline of 9 July 2019.

The registration dossier was updated with the 90-day inhalation study on 29 October 2019. At the request of the eMSCA, the registrants provided the original study report of the sub-chronic toxicity study by inhalation performed with two (rather than the four requested by the eMSCA) forms of pyrogenic SAS in rats in May 2020 and provided background information in a meeting with the eMSCA. This study report formed the primary basis for the evaluation.

Other information considered in the evaluation included the study by Reuzel *et al.* (1991) and the reanalysis of these data by Weber *et al.* (2018). Additionally, the RAC opinion of the closely related substance silanamine; 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica; pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide (EC number 272-697-1, CAS RN 68909-20-6) of 5 December 2019 was taken into account (RAC, 2019).

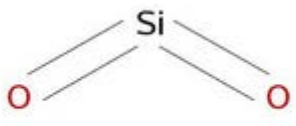
7.3. Identity of the substance

Table 5

SUBSTANCE IDENTITY	
Public name:	Silicon dioxide
EC number:	231-545-4
CAS number:	7631-86-9
Index number in Annex VI of the CLP Regulation:	-
Molecular formula:	SiO_2
Molecular weight range:	60-60.2 g/mol
Synonyms:	Silica Synthetic amorphous silica Amorphous silica Dioxosilane Fumed silica Kieselgel Silica gel Precipitated amorphous silica

Type of substance x Mono-constituent ☐ Multi-constituent ☐ UVCB

Structural formula:



7.4. Physico-chemical properties

Table 7

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Solid: particulate / powder, white, odourless, inorganic
Vapour pressure	Silicon dioxide/SAS does not have a measurable vapour pressure under normal conditions.
Water solubility	Water solubility of all non surface-treated SAS products (silica gel, colloidal, precipitated and pyrogenic SAS) is in the range of 100 mg/L or higher. Applying a modified method to accomplish sufficient material wetting, all hydrophobic SAS products analysed (surface treated pyrogenic SAS only) exhibit a solubility between 100 and 160 mg/L in 10 % ethanol/water. The registrant states that it is expected, that other SAS products not tested so far will fit into that range. Thus, the registrant concludes that the solubility of

	hydrophobic SAS (surface-treated SAS) products does not differ from the results of hydrophilic SAS (non-surface-treated SAS). Questions from the eMSCA to provide physico-chemical information from the various types were annulled in an appeal case (BoA, 2017).
Partition coefficient n-octanol/water (Log Kow)	NA
Flammability	non flammable
Explosive properties	non explosive
Oxidising properties	no
Granulometry	The SAS particle size structure of SAS for the dry forms (silica gel, precipitated and pyrogenic SAS) have to be distinguished into three bottom up particular systems constituent particles, aggregates and agglomerates while colloidal silica is a monodisperse system in rare cases poly-disperse consisting of the constituent particles only. Typical ranges for the SAS particular systems: · Constituent (primary) particles: Size range 1 to 100 nm, with mostly spherical form. · Aggregates: Size range >100 nm to 5 mm, depending on the form of SAS. · Dry powders typically form agglomerates while forming large loose structures of aggregates, with van der Waals and H-bridges bonds between the aggregate surfaces, which can be easily destroyed towards the forming aggregates by inducing low shear forces. Typical agglomerate size of SAS powders can reach several hundred micrometres.
Stability in organic solvents and identity of relevant degradation products	Silica gel is an inorganic silicon oxide. It does not dissolve in any organic solvent.
Dissociation constant	The overall reaction for dissolution of (solid) silica to monosilicic acid, and reverse precipitation, i.e. polymerisation and de-polymerisation, is given by the following equation. SiO2 + 2 H2O = Si(OH)4. Orthosilicic acid (H4SiO4) has a reported pKa value of 9.84 for the dissociation of one proton and a pKa2 of13.2 for the second proton removal at 25 °C

NA: Not Applicable

7.5. Manufacture and uses

7.5.1. Quantities

Table 8

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☐ 10 – 100 t	☐ 100 – 1000 t	☐ 1000- 10,000 t	☐ 10,000-50,000 t

☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☒ > 1000,000 t	☐ Confidential

7.5.2. Overview of uses

ECHA dissemination web site:

SAS is used by consumers, in articles, by professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing.

Biocidal Uses:

The substance synthetic amorphous silica gel obtained by wet-process, with the CAS RN 112926-00-8 (which includes both “precipitated silica” and “silica gel”) and EC number 231-545-4 is approved for use as a biocide in the EEA and/or Switzerland, for: controlling insects, ants, etc.

Table 9

USES Use(s)	
Uses as intermediate	
Formulation	This substance is used in the following products: polymers, coating products, polishes and waxes, non-metal-surface treatment products and inks and toners. Release to the environment of this substance can occur from industrial use: formulation of mixtures and formulation in materials.
Uses at industrial sites	This substance is used in the following products: adsorbents, fillers, putties, plasters, modelling clay, coating products, pH regulators and water treatment products, polymers, non-metal-surface treatment products and metal surface treatment products. This substance is used in the following areas: formulation of mixtures and/or re-packaging and agriculture, forestry and fishing. This substance is used for the manufacture of: chemicals and textile, leather or fur. Release to the environment of this substance can occur from industrial use: in the production of articles, in processing aids at industrial sites, as an intermediate step in further manufacturing of another substance (use of intermediates), as processing aid, as processing aid and of substances in closed systems with minimal release.
Uses by professional workers	This substance is used in the following products: adsorbents, coating products, adhesives and sealants, pH regulators and water treatment products and non-metal-surface treatment products. This substance is used in the following areas: health services, formulation of mixtures and/or re-packaging and agriculture, forestry and fishing. This substance is used for the manufacture of: chemicals and textile, leather or fur. Other release to the environment of this substance is likely to occur from: indoor use (e.g. machine wash liquids/detergents, automotive care products, paints and coating or adhesives, fragrances and air fresheners), outdoor use, indoor use in close systems with minimal release (e.g. cooling liquids in refrigerators, oil-based electric heaters) and outdoor use in close

	systems with minimal release (e.g. hydraulic liquids in automotive suspension, lubricants in motor oil and break fluids).
Consumer Uses	This substance is used in the following products: coating products, inks and toners, fillers, putties, plasters, modelling clay, polishes and waxes, adhesives and sealants and cosmetics and personal care products. Other release to the environment of this substance is likely to occur from: indoor use (e.g. machine wash liquids/detergents, automotive care products, paints and coating or adhesives, fragrances and air fresheners) and outdoor use.
Article service life	Release to the environment of this substance can occur from industrial use: industrial abrasion processing with low release rate (e.g. cutting of textile, cutting, machining or grinding of metal), of articles where the substances are not intended to be released and where the conditions of use do not promote release and industrial abrasion processing with high release rate (e.g. sanding operations or paint stripping by shot-blasting). Other release to the environment of this substance is likely to occur from: indoor use in long-life materials with low release rate (e.g. flooring, furniture, toys, construction materials, curtains, foot-wear, leather products, paper and cardboard products, electronic equipment) and outdoor use in long-life materials with low release rate (e.g. metal, wooden and plastic construction and building materials). This substance can be found in complex articles, with no release intended: machinery, mechanical appliances and electrical/electronic products e.g. refrigerators, washing machines, vacuum cleaners, computers, telephones, drills, saws, smoke detectors, thermostats, radiators, large-scale stationary industrial tools) and Vehicles (e.g. personal vehicles, delivery vans, boats, trains, metro or planes)). This substance can be found in products with material based on: stone, plaster, cement, glass or ceramic (e.g. dishes, pots/pans, food storage containers, construction and isolation material), plastic (e.g. food packaging and storage, toys, mobile phones), metal (e.g. cutlery, pots, toys, jewellery) and wood (e.g. floors, furniture, toys).

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

SAS does not have a harmonised classification.

7.6.2. Self-classification

• In the registration(s):

No classification is indicated in the substance dossier for any of the following types:

- Synthetic amorphous silica, nanostructured
- Silica gel, precipitated
- Precipitated amorphous silica
- Silica, amorphous, fumed, crystalline-free

• The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory:

Skin irrit. 2, H315

Eye irrit. 2, H319

STOT SE 3 (respiratory tract irritation), H335

Acute Tox. 4, H332
STOT RE 1 (lungs) (inhalation), H372
STOT RE 2 (lungs) (inhalation), H373
Aquatic Chronic 3, H412
Flam. Liq. 2, H225
Asp. Tox. 1, H304
Muta. 1B (inhalation), H340
Carc. 1A (inhalation), H350
Carc. 1B (inhalation), H350
Water-react. 1, H260
Water-react. 3, H261

7.7. Environmental fate properties

Not evaluated

7.8. Environmental hazard assessment

Not evaluated

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

Not evaluated

7.9.2. Acute toxicity and Corrosion / Irritation

Not evaluated

7.9.3. Sensitisation

Not evaluated

7.9.4. Repeated dose toxicity

Relevant background information

The original concern was for the effects of sub-chronic inhalation exposure to SAS and in particular the lack of knowledge on the relationship between particle characteristics and toxic potency. This concern was primarily based on Reuzel *et al.* (1991) who performed a 13-week inhalation study with three different SAS forms (untreated pyrogenic SAS, i.e. Aerosil 200, surface-treated pyrogenic SAS, i.e. Aerosil R 974, and precipitated SAS, i.e. Sipernat 22S). Differences in the toxicity profile were clearly demonstrated, with the main difference in the incidence of focal interstitial fibrosis. Rats were exposed to 1, 6 or 30 mg Aerosil 200/m^3, to 30 mg Sipernat 22S/m^3 or to 30 mg surface-treated Aerosil R 974/m^3. Separate exposure groups were included for recovery periods of 13, 26, 39 and 52 weeks. A low incidence of fibrosis was observed 13 weeks post-exposure in rats exposed to Sipernat 22S and to Aerosil R 974; at 26 weeks post-exposure fibrosis was observed in 0/10 rats exposed to Sipernat 22S and in 1/10 rats exposed to Aerosil R 974. No fibrosis was observed after 39 and 52 weeks.

Of the three forms, Aerosil 200 is the most comparable to the SAS forms used in the new 90-day inhalation study that was performed within this Substance Evaluation (SEV) process (Anonymous, 2019). In the Aerosil 200 exposure groups higher incidences of fibrosis were observed (seen as amorphous eosinophilic, collagen-containing thickenings of the septa) which were very consistent, showed a clear concentration-response relationship and were still observed after 52 weeks recovery (see Table 10 for details). Also the lung collagen content showed a dose dependent increase which was more pronounced in males. The lung collagen content gradually decreased over time, but at 6 and 30 mg/m^3 it did not return to control levels within the 52 week recovery period, indicating that the observed fibrosis is not completely reversible. Other effects induced by Aerosil 200 in both sexes included

an accumulation of alveolar macrophages, IPLI (intra-alveolar polymorphonuclear leucocytic infiltration), and increased septal cellularity. Alveolar bronchiolisation was only observed in males and reversable after 39 weeks.

Table 10: Summary Aerosil 200 focal interstitial fibrosis, from the study report by Reuzel *et al.* (1991), including severity data from the study report

Dose (mg/m^3)	At end of treatment		13 wks recovery		26 wks recovery		39 wks recovery		52 wks recovery	
	M	F	M	F	M	F	M	F	M	F
0	0/10	0/10	0/5	0/5	0/5	0/5	0/5	0/5	0/10	0/10
1	0/10	0/10	0/5	1/5	0/5	1/5	0/5	0/5	0/10	1/10
Very slight				1		1				1
Slight										
Moderate										
6	0/10	0/10	2/5	1/5	3/5	2/5	3/5	1/5	2/10	1/10
Very slight			1	1	3	2	3	1	2	1
Slight			1							
Moderate										
30	0/10	0/10	5/5**	4/5*	4/5*	5/5**	5/5**	4/5*	10/10**	10/10**
Very slight				1	1	4		2	8	9
Slight				3	2	1	5	2	2	1
Moderate			5		1					

*p<0.05
**p<0.01

The pathology slides of Reuzel *et al.* (1991) have been re-stained with hematoxylin-eosin (HE) staining and re-evaluated almost 30 years later, the result of which was published by Weber *et al.* (2018). Only slides of males at time points 0, 13 weeks and 52 weeks recovery were still available. The diagnostic criteria and terminology used throughout the study were based upon recognised texts and current scientific literature, that is, according to International Nomenclature and Harmonization of Diagnostic Criteria (INHAND) nomenclatures.

Fibrogenesis is defined by Weber *et al.* in this re-evaluation as “Increases in septal or interstitial thickness resulting from edema or inflammation without substantial fibre cross-linking. In the present study associate with minimal inflammatory infiltration considered to be fully reversible”.

Fibrosis is defined by Weber *et al.* as “Observable increase in amount or abnormal location of collagen in lung parenchyma, resulting in disruption of the normal lung architecture. Occurrence in alveolar septa, interstitium, and pleura. Formation of distinct collagen bands”

In this re-evaluation Weber *et al*. concluded that only single incidences of minimal focal fibrosis were observed, without relation to the concentration, and a slight increase in fibrogenesis at the high dose males (2/10). There was also an increase in inflammation indicators, comparable with the other effects noted by Reuzel *et al.* (1991).

In light of this assessment and in particular regarding the interpretation of the Reuzel study and its re-evaluation by Weber *et al.*, the recent RAC opinion of silanamine should also be mentioned (RAC, 2019). In the CLH evaluation of silanamine a read-across with Aerosil R 974 (surface-treated pyrogenic SAS modified with Dimethyldichlorosilane (DDS)) was

used, which is structurally similar to silanamine and shares physical, chemical and toxicological properties. RAC noted several issues with Weber *et al*.:

- the re-evaluation did not concern all animals, and only one lung section per animal;

- the almost 30-year old slides were de-cover-slipped, re-stained (with standard hematoxylin and eosin staining) and then cover-slipped again, whereby the de-cover-slipping may potentially have damaged the original tissue samples;

- the specific Van Gieson stain for the detection of collagen was not used in the re-evaluation nor was OH-proline measured;

- the claimed recovery pertains to unusually long recovery periods for a 13-week rat study (13-52 weeks, as compared to 4 weeks as recommended in the OECD test guideline).

Moreover, it was noted by RAC that although exposure-related fibrogenesis and structural remodelling of the lung tissue may be reversible, they cannot be excluded as an adverse effect that could progress to fibrosis, if exposure persists and in the presence of another detrimental pathology, such as infection. In all cases, histopathological findings like these could account for clinical symptoms of respiratory distress and were considered relevant for classification as STOT RE.

The new 90-day inhalation study (Anonymous, 2019)

Methodology

The study performed as part of the SEv is a 90-day nose-only inhalation study in rats (GLP and according to OECD TG 413) with recovery periods of 0, 3, 6, and 12 months. Two forms of SAS were tested, both pyrogenic SAS with EC number 231-545-4, but different in surface area. SAS 1 had a high surface area of approximately 400 m^2/g and SAS 2 a lower surface area of 40-50 m^2/g. The doses included clean air control, and SAS 1/SAS 2 at nominal concentrations of 0.5 mg/m^3, 1 mg/m^3, 2.5 mg/m^3 and 5 mg/m^3 for 6 hours/day and 5 days/week. Aerosol concentrations were measured in all treated groups gravimetrically by filter samples and mean concentrations were very close to the target concentration for all dose groups. The number of animals allocated to each group was 10 rats/sex/dose for the groups without recovery and 5 rats/sex/dose for the recovery groups. Analyses included gross pathology of all organs, histopathology of the respiratory organs including lymph nodes, bronchoalveolar lavage (BAL) and collagen analysis of the lung tissue. Silica content of the lung associated lymph nodes was determined at the end of treatment and end of the recovery period by EDX.

Some remarks regarding the study protocol:

1) The dose levels chosen were low when compared with the other studies, in particular Reuzel *et al.* (1991), and also compared to the guidance values for classification for repeated dose inhalation toxicity (below 20 mg/m^3 for STOT RE Cat. 1 and between 20 and 200 mg/m^3 for Cat. 2). It is stated by the registrant that they were based on a 90-day range-finding study with another form of SAS (precipitated), but this study is not available.

2) Originally four groups of SAS were requested:

   i. the lowest specific surface area with the lowest number of hydroxyl groups,

   ii. the lowest specific surface area with the highest number of hydroxyl groups,

   iii. the highest specific surface area with the lowest number of hydroxyl groups,

   iv. the highest specific surface area with the highest number of hydroxyl groups,

   Only two SAS forms were tested, representing the lowest and highest specific surface area. The reason given by the registrant not to test forms with different numbers of hydroxyl groups was that the effective OH concentration is independent of the specific surface area and also independent of the manufacturer. The silanol surface density of the two SAS tested was practically the same as determined by thermogravimetric analysis.

3) The one-year recovery group was not included in the SEv request but added by the registrant to investigate the reversibility of the effects. This long recovery delayed the REACH registration update and the availability of the study report.

4) Haematology, clinical chemistry and ophthalmoscopy were not performed, as these were excluded in the ECHA decision.

5) The definitions of fibrosis and fibrogenesis used were the same as those in the study by Weber *et al.* (2018) (see previous page).

Results

Two animals died during the study and two were killed in moribund condition. All were from different exposure groups and the deaths were not treatment related.

There were no statistically significant changes in body weight or food consumption in any of the treated groups. In gross pathology, enlarged lung-associated lymph nodes (LALN) were observed in the SAS 1 mid and high dose groups and in all SAS 2-treated groups. SAS 1 induced a statistically significant increase of the absolute and relative lung wet weights in the female high dose group at 1 day post-exposure only. At 3 months post-exposure, this effect had disappeared. SAS 2 induced statistically significant increases of the absolute and relative lung wet weights in the low, mid and high dose groups at 1 day post-exposure (both sexes). Lung weights recovered at 3 months post-exposure; the high dose group only showed a persistent statistically significant increase in lung weight at 6 and 12 months post-exposure.

BAL measurements showed at day 1 post-exposure statistically significant increases of polymorphonuclear neutrophils (PMN) in the SAS 1 mid and high dose groups of both sexes. In both dose groups a full recovery was detected at 3 months post-exposure. At day 1 post-exposure statistically significant increases of PMN were detected in all SAS 2 dose groups of both sexes. Full recovery was detected in the very low dose group at 3 months, in the low dose group at 6 months and in the mid and high dose groups at 12 months post-exposure. For lactic dehydrogenase (LDH), ß-glucuronidase (GLU) and total protein (TP) no statistically significant increases were detected in all SAS 1 groups at all 4 sacrifice dates (but for total protein in the female SAS 1 high dose group at day 1). In the SAS 2 mid- and high-dose groups, statistically significant increases of LDH, GLU and TP were observed at 1 and 90 days post-exposure; these effects returned to normalisation mostly at 6 and 12 months post-exposure.

Hydroxyproline as an indicator of collagen in lungs was statistically significantly increased in the high dose males of the SAS 2 group after 12 months recovery.

Silica content measurements of the lymph nodes showed no dose-response relationship and very high variation.

In the histopathological evaluation, treatment-related findings were noted in nasal cavities, lungs, and lung associated lymph nodes.

In nasal cavities, the major lesions consisted of:

- Slight mucosal degeneration in the high dose groups at the end of treatment

- Goblet cell proliferation in levels 1 and 2 and nasopharyngeal duct at the end of treatment and after 13 weeks recovery in all SAS 1 and SAS 2 groups.

- Hyaline inclusions in olfactory mucosa at higher incidences and severity with increased incidences during the course of the study.

- Chitinase-positive crystals in olfactory mucosa in nasal cavity levels 2-4 up to 26-week recovery without any further injury in olfactory mucosa, mainly in SAS 1 treated animals.

In lungs, the findings consisted of:

- *End of treatment*: discoloration or discoloured foci in lungs from animals treated at ≥ 1.0 mg/m^3 SAS 2 associated with inflammatory lesions that increased in incidence and/or severity in test item-treated groups.

- increased perivascular infiltration in SAS 1 groups ≥ 1.0 mg/m^3 and all SAS 2-treated groups.

- increased alveolar macrophages and macrophage aggregations, as well as macrophage type II hyperplasia dose-dependently for SAS 1 and SAS 2 associated with interstitial inflammation, granulomas at the bronchio-alveolar junctions, granulomatous inflammation at a minor severity was noted in single animals from the very low and low dose (SAS 1), and in most animals from mid and high dose groups (SAS 1) and all dose groups of SAS 2.

- bronchio-alveolar hyperplasia in single animals from SAS 1 groups ≥ 1.0 mg/m^3 and all SAS 2 groups.

- hyperplasia in the BALT (Bronchus Associated Lymphoid Tissue) in one 0.5 mg/m^3 SAS 2 male and one group 2.5 mg/m^3 SAS 2 female.

- minimal macrophage agglomeration in the BALT of a few animals at ≥ 1.0 mg/m^3 SAS 1 increasing to almost all animals at 5.0 mg/m^3 SAS 1, as well as in almost all animals treated with SAS 2. Granulomatous inflammation in the BALT in animals treated with SAS 2.

- BALT fibrogenesis in single animals at 0.5 and 1.0 mg/m^3 SAS 1, at an increased incidence at higher doses of SAS 1, and in all doses of SAS 2 with increasing incidence.

- *13 weeks recovery*: in macroscopic analysis discoloured foci in the lungs mainly in groups treated with SAS 2.

- increased perivascular infiltration in SAS 2-treated groups.

- increased in incidence and severity of alveolar macrophages in SAS 2-treated groups and macrophage aggregates in animals at ≥ 1.0 mg/m^3 SAS 1 and in all groups treated with SAS 2.

- Increased incidence of macrophage type II hyperplasia and interstitial inflammation in SAS 2-treated groups and single cases of granulomas at the alveolar-bronchiolar junctions in SAS 1-treated groups.

- Increased alveolar-bronchiolar hyperplasia in SAS 2-treated groups without clear dose-dependency.

- BALT macrophage agglomeration was noted in a few animals from SAS 2 groups associated with some cases of granulomatous inflammation. The latter caused fibrogenesis in the BALT in single cases of SAS 2-treated animals and in single cases at ≥ 1.0 mg/m^3 SAS 1.

- Fibrogenesis due to inflammatory processes in one animal per sex at 0.5 mg/m^3 SAS 1, one female at 2.5 mg/m^3 SAS 1, but in most animals treated with SAS 2.

- *26 weeks recovery*: similar findings as observed after 13 weeks recovery.

- *52 weeks recovery*: still increased discoloured foci in lungs from animals at ≥ 1.0 mg/m^3 in SAS 2-treated groups.

- SAS 1-treated groups: no findings except the presence of macrophage agglomeration.

- SAS 2-treated groups: still a few inflammatory lesions present, mainly in animals ≥ 1.0 mg/m^3.

- SAS 2-treated groups: no BALT inflammation present any longer, however, in a few animals at 2.5 and 5.0 mg/m^3, there was a minimal BALT macrophage agglomeration.

- SAS 2-treated groups: no BALT inflammation present any longer, however, in a few animals at 2.5 and 5.0 mg/m^3, there was a minimal BALT macrophage agglomeration, and increased incidence and/or severity of macrophages in animals from ≥ 1.0 mg/m^3 SAS 2.

- SAS 2-treated groups: fibrogenesis in the lungs at increased incidence in both sexes at 2.5 and 5.0 mg/m^3 SAS 2 likely due to still ongoing inflammatory processes, and minimal interstitial fibrosis in one animal at 5.0 mg/m^3.

In lymph nodes, the findings consisted of:

- *End of treatment*:

- granulomas in lymph nodes >0.5 mg/m^3 SAS 1 and in all SAS 2 groups

- related granulomatous inflammation at a minor severity in single males 2.5 and 5.0 mg/m^3 SAS 1 but in a high number of animals from all groups treated with SAS 2

- lymphoid hyperplasia in most affected lymph nodes

- fibrogenesis in the lymph nodes from several animals from all SAS 2-treated groups, and fibrosis in one female at 0.5 mg/m^3 SAS 2, and in both sexes at ≥ 1.0 mg/m^3 SAS 2.

- *13 weeks recovery*:

- granulomas and single cases of granulomatous inflammation in animals at >0.5 mg/m^3 SAS 1, and at all doses of SAS 2,

- fibrogenesis in one female at 2.5 mg/m^3 SAS 1, and at fibrogenesis/fibrosis at a higher incidence at minor severities in all SAS 2 groups.

- *26 weeks recovery*:

- SAS 1: only single cases of lymphoid hyperplasia and granulomas in a few animals at 2.5 and 5.0 mg/m^3 in SAS 1

- SAS 2: increased in incidence and severity of lymphoid hyperplasia in all SAS 2 groups and granulomas in all SAS 2 groups

- SAS 2: granulomatous inflammation and dose-dependent increased severity of fibrogenesis and fibrosis.

- *52 weeks recovery*:

- SAS 2: granulomas or granulomatous inflammation at all dose levels

- SAS 2: increased incidence of lymphoid hyperplasia in animals at >1.0 mg/m^3

- SAS 2: fibrogenesis or fibrosis in a few animals of all SAS 2 groups, with high incidence at 5 mg/m^3.

Conclusion repeated dose toxicity

In the new 90-day inhalation study, the most serious effects induced by both SAS materials were interstitial inflammation, granuloma, fibrogenesis, and fibrosis of the lungs and lymph nodes. There was a clear link between particle size and the severity and persistence of the effects, with higher incidence, severity and duration associated with larger particles. The LOAEC of SAS 1 was 1 mg/m^3, while SAS 2 induced effects at all tested doses, the lowest of which was 0.5 mg/m^3.

Regarding granuloma and fibrogenesis, SAS 1 induced a concentration dependent increase in granuloma in the lungs with recovery after 6 months and in the lymph nodes with recovery after 12 months. A dose dependent increase in the incidence of fibrogenesis was observed in the lungs at the end of treatment with SAS 1. SAS 2 induced high incidences (50-100%) of granuloma and fibrogenesis in the lungs at all dose levels. These effects as well as fibrosis were also observed with high incidence in the lymph nodes, although with some strange negatives, which might be caused by technical difficulties due to the small tissue. There was some recovery after 12 months, but this was incomplete, in particular for the higher dose levels.

Other effects included some incidences of mucosal degeneration in the nose, as well as goblet cell proliferation and hyaline inclusions in the nasal cavity. In the lung, alveolar histiocytosis, macrophage aggregations, secondary alveolar and BALT hyperplasia indicative for exposure to particulate material were observed.

The effects observed in the new study are in line with the effects observed by Reuzel *et al. (1991)* for pyrogenic SAS at 6 mg/m^3, albeit a direct comparison is hampered by the difference in terminology of the histopathology. In the discussion of the Reuzel results,

RAC noted that fibrosis/fibrogenesis at the lower exposure concentrations of pyrogenic SAS (1 and 6 mg/m^3) cannot be attributed to just particle (over)load of the lungs. Moreover, the classification of surface treated SAS was based on inflammation of lung tissue (main mechanism of toxicity identified), associated with a morphological tissue reaction (hypertrophy, lung injury, partial hyperplasia of the bronchiolar epithelium, collagen remodelling). Similar effects are observed in the new 90-day study at lower concentrations (surface treated SAS was only tested at 30 mg/m^3 by Reuzel *et al.*). Although the severity and incidence of in particular SAS 1 was relatively minor for many effects, it should also be considered that the highest dose tested (5 mg/m3 or 0,005 mg/L) is a factor 4 below the guidance value for classification in Cat 1 for STOT RE (<0,02 mg/L).

The purpose of the new 90-day inhalation study was to investigate the concern for repeated dose inhalation toxicity of SAS. The outcome of the study and in particular the dose-dependent increase in granuloma and fibrosis/fibrogenesis observed in the lungs and lymph nodes confirm the concern. The effects were observed even though the dose levels used were very low and were not reversible within a 12 month recovery period. Although no SAS groups with different numbers of hydroxyl groups were tested, the outcome of the study gives sufficient information to reach a conclusion.

The eMSCA concludes that there is sufficient ground to start the process for harmonised classification and labelling for the endpoint specific target organ toxicity - repeated dose toxicity (STOT RE) (lung/respiratory tract).

7.9.5. Mutagenicity

Not evaluated

7.9.6. Carcinogenicity

Not evaluated

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

Not evaluated

7.9.8. Hazard assessment of physico-chemical properties

Not evaluated

7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

7.9.10. Conclusions of the human health hazard assessment and related classification and labelling

The eMSCA concludes that there is sufficient ground to start the process for harmonised classification and labelling for the endpoint repeated dose toxicity.

7.10. Assessment of endocrine disrupting (ED) properties

Not applicable

7.11. PBT and VPVB assessment

Not applicable

7.12. Exposure assessment

Not applicable

7.13. Risk characterisation

Not Applicable

7.14. References

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7.15. Abbreviations

BAL	Bronchoalveolar lavage
BALT	Bronchio-associated lymphoid tissue
LOAEC	Lowest observed adverse effect concentration
OECD	Organization for Economic Cooperation and Development
PMN	Polymorphonuclear neutrophils
RAC	Committee for Risk Assessment
SAS	Synthetic amorphous silica
STOT RE	Specific Target Organ Toxicity Repeated Exposure