SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 23 November 2017

doi: 10.2903/j.efsa.2018.5088

Re-evaluation of silicon dioxide (E 551) as a food additive

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Abstract

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) provides a scientific opinion re-evaluating the safety of silicon dioxide (E 551) when used as a food additive. The forms of synthetic amorphous silica (SAS) used as E 551 include fumed silica and hydrated silica (precipitated silica, silica gel and hydrous silica). The Scientific Committee on Food (SCF) established a group acceptable daily intake (ADI) 'not specified' for silicon dioxide and silicates. SAS materials used in the available biological and toxicological studies were different in their physicochemical properties; their characteristics were not always described in sufficient detail. Silicon dioxide appears to be poorly absorbed. However, silicon-containing material (in some cases presumed to be silicon dioxide) was found in some tissues. Despite the limitations in the subchronic, reproductive and developmental toxicological studies, including studies with nano silicon dioxide, there was no indication of adverse effects. E 551 does not raise a concern with respect to genotoxicity. In the absence of a long-term study with nano silicon dioxide, the Panel could not extrapolate the results from the available chronic study with a material, which does not cover the full-size range of the nanoparticles that could be present in the food additive E 551, to a material complying with the current specifications for E 551. These specifications do not exclude the presence of nanoparticles. The highest exposure estimates were at least one order of magnitude lower than the no observed adverse effect levels (NOAELs) identified (the highest doses tested). The Panel concluded that the EU specifications are insufficient to adequately characterise the food additive E 551. Clear characterisation of particle size distribution is required. Based on the available database, there was no indication for toxicity of E 551 at the reported uses and use levels. Because of the limitations in the available database, the Panel was unable to confirm the current ADI 'not specified'. The Panel recommended some modifications of the EU specifications for E 551.

Keywords: silicon dioxide, silica, SAS, E 551, CAS 7631-86-9, CAS 112945-52-5, CAS 112926-00-8

Requestor: European Commission

Question number: EFSA-Q-2011-00576

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Acknowledgements: The ANS Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

Suggested citation: EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), Younes M, Aggett P, Aguilar F, Crebelli R, Dusemund B, Filipič M, Frutos MJ, Galtier P, Gott D, Gundert-Remy U, Kuhnle GG, Leblanc J-C, Lillegaard IT, Moldeus P, Mortensen A, Oskarsson A, Stankovic I, Waalkens-Berendsen I, Woutersen RA, Wright M, Boon P, Chrysafidis D, Gürtler R, Mosesso P, Parent-Massin D, Tobback P, Kovalkovicova N, Rincon AM, Tard A and Lambré C, 2018. Scientific Opinion on the re-evaluation of silicon dioxide (E 551) as a food additive. EFSA Journal 2018;16(1):5088, 70 pp. https://doi.org/10.2903/j.efsa.2018.5088

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

Silicon dioxide (E 551) is authorised as a food additive in the European Union (EU) in accordance with Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives and specific purity criteria are defined in the Commission Regulation (EU) No 231/2012.

In this opinion, the Panel did not consider data obtained with crystalline silica (an IARC class 1 carcinogen by inhalation) because only the amorphous form of silicon dioxide (synthetic amorphous silica (SAS)) is authorised as a food additive.

According to the EU specifications for silicon dioxide (E 551), the forms of SAS used as a food additive E 551 include fumed (pyrogenic) silica and hydrated silica (precipitated silica, silica gel and hydrous silica) depending on the process (thermal or wet) used for their manufacture. The Panel noted that among the types of SAS (i.e. silica gel, precipitated silica, pyrogenic (fumed) silica and colloidal silica (silica sol)), colloidal silica is not authorised as a food additive (E 551).

The food additive, silicon dioxide (E 551), is a material comprised of aggregated nanosized primary particles. These aggregates can further agglomerate to form larger structures. The sizes of the aggregates and agglomerates are normally greater than 100 nm. However, depending on the starting material and/or on the manufacturing process, it cannot be totally excluded that some aggregates of primary particles could be smaller than 100 nm in size.

The Panel noted that several analytical methods are available to measure the particle size of nanomaterials (dynamic light scattering (DLS), laser diffraction (LD), transmission electron microscopy (TEM), scanning electron microscopy (SEM)). These methods measure different particle characteristics, which are reflected in the different numerical size values obtained.

The Panel noted that in some biological and toxicity studies (especially those conducted in the 1960–1970s), while the authors reported analysis of 'silica' content, analytical methods available at the time were only capable of measuring silicon. The Panel considered that while this was expressed as silica by the authors, it was not possible to determine whether it was silica or silicon that was measured. The Panel noted that the analysis of silicon cannot distinguish between silicon from the food additive E 551, natural presence of silicon, or silicon from other sources of silicon dioxide.

In addition, a number of studies were available with chemically modified SAS particles such as some of those used by the pharmaceutical industry. These studies were not included in the present assessment as this material was clearly different from silicon dioxide (E 551) used as a food additive.

In the few studies available in animals, after oral administration of fumed or precipitated SAS, the silicon content of the liver and kidney, and occasionally in the spleen was slightly increased. Studies in rats indicated no accumulation of silicon in animals after repeated oral applications of SAS. In humans, there was little indication of absorption of SAS after ingestion; however, silicon dioxide (of unknown origin) was occasionally found in human liver and spleen tissues. Some studies reported that less than 0.5% of silicon orally applied as silicon dioxide (1,250 mg) was excreted via urine but urinary silicon was always within the range of normal physiological variation.

There was evidence for a low acute oral toxicity of SAS and for low toxicity; after repeated oral administration of SAS, no adverse effects were detected even at high dose levels up to 9,000 mg/kg body weight (bw) per day. Silicon dioxide (E 551) as a food additive did not raise a concern with respect to genotoxicity.

For SAS used as a food additive, the available *in vitro* and *in vivo* study results, although of limited relevance did not indicate any potential for genotoxicity and overall the Panel considered that SAS used as a food additive did not raise a concern with respect to genotoxicity.

There were some indications for induction of structural and/or numerical chromosomal aberrations *in vitro* for SAS not used as a food additive nor used in either cosmetics or pharmaceuticals. These results were not considered relevant by the Panel for the re-evaluation of silicon dioxide (E 551), since this material is not used as a food additive. However, the Panel noted that their presence in the food additive cannot be excluded due to a lack of precision in the specifications for E 551.

A long-term feeding study in rats and in mice indicated that SAS was not carcinogenic; however, the precise characteristics of the test material were not well reported, in particular the description of the primary particle size.

No reproductive toxicity was noted after treatment with SAS; however, the validity of these results was limited since only one dose of 500 mg/kg bw per day was tested and the group size of pregnant rats was small. Prenatal developmental toxicity studies with silica gel showed no developmental effects up to the highest doses tested (1,350 mg/kg bw per day in rats and 1,600 mg/kg bw per day in hamsters).

Overall, the Panel noted that the SAS test items used in the biological and toxicological studies available were different in their physicochemical properties (e.g. particle size distribution). In addition, the characteristics of the test materials were not always described in sufficient detail. Given the absence of information about the particle size distribution for silicon dioxide (E 551) in the current EU specifications, the Panel considered that no SAS preparation used in any single study might be fully representative of the food additive E 551. Accordingly, the Panel considered that one major uncertainty in the risk assessment of silicon dioxide (E 551) was that different characteristics of the various SAS forms may affect their behaviour. The variety of SAS in line with the specifications and currently on the market can result in differences in surface properties and in the absorption of silicon dioxide (E 551). Nevertheless, despite the limitations of the toxicological database available with SAS samples closely related to the food additive E 551, there was no indication of adverse effects. However, the absence of a robust long-term study with a well-characterised food additive and following the current guidelines remained an uncertainty.

Because nanoparticles of silicon dioxide are present in the food additive E 551, studies performed with specifically designed engineered nano silicon dioxide were included in this assessment in order to assess any toxicity associated with nanoparticles present in the food additive, provided they were performed using amorphous silicon dioxide. The Panel noted that although these nanomaterials are not intended to be used as a food additive E 551, the current EU specifications for E 551 would authorise their utilisation as such.

The Panel noted that data obtained with nano silicon dioxide designed for specific purposes, which tend to markedly change their surface properties, must be interpreted cautiously as regards their relevance for evaluating possible effects of the food additive E 551. Notwithstanding all the aforementioned considerations and evaluations, the Panel considered that to date, no adverse effects have been observed with nano-SAS in the available oral toxicity studies *in vivo*.

The dietary exposure to silicon dioxide (E 551) from its use as a food additive was calculated according to different scenarios. The Panel did not identify brand loyalty to a specific food category, and therefore, considered that the non-brand-loyal scenario covering the general population. This approach was considered the most appropriate and realistic scenario for risk characterisation of E 551 because it is assumed that the population is most likely to be exposed long-term to the food additive E 551 present at the mean reported use in processed food. The exposure assessments were hampered by several uncertainties. Overall, it was considered that the exposure was overestimated due to the use levels used and assumptions made in the exposure assessment. For an elaborate discussion of the uncertainties, see Section 3.4.5.

The Panel noted that the highest exposure estimates were always much lower than the no observed adverse effect levels (NOAELs) identified in the different toxicity studies available.

Considering that:

- the EU specifications for silicon dioxide (E 551) allow for the use of SAS with various physicochemical properties,
- depending on the method used for the analytical determination of particles of silicon dioxide (including the preparation of the sample; e.g. ultrasonication), the presence of particles in the nano-range in food and biological samples has been reported in very variable amounts,
- 'primary particles' of silicon dioxide (E 551) aggregate during the production process. The resulting aggregates (which may be in the nano-range or larger) further agglomerate in foods and/or when in contact with biological fluid,
- there is an uncertainty about the extent to which disagglomeration and/or release of primary nanoparticles of SiO_2 may occur from such agglomerates after ingestion of food containing the food additive (E 551),
- nanoparticles of SAS interact with various components of a biological milieu and are covered by a corona with a composition that is variable from one preparation to another,
- the highest exposure estimates (50 mg/kg bw per day) were always much lower (at least one order of magnitude) than the NOAELs identified (the highest doses tested) in the different toxicity studies available,
- due to the analytical techniques used and in the absence of clear information, it was not always possible to determine whether it was silica (SiO_2) or silicon (Si) that was measured in the biological samples,
- silicon dioxide appears to be poorly absorbed; however, silicon containing material (in some cases presumed to be silicon dioxide) was found in some tissues,

- the toxicological data available with SAS samples closely related to the food additive E 551 were used for the evaluation of the food additive,
- despite the limitations in the subchronic, reproductive and developmental toxicological studies, including studies with nano silicon dioxide, there was no indication of adverse effects,
- SAS used as a food additive does not raise a concern with respect to genotoxicity,
- there are some indications for genotoxicity for SAS not reported to be used as a food additive, in cosmetics or pharmaceuticals and for intentionally engineered nano-SAS. These results were not considered relevant by the Panel for the re-evaluation of silicon dioxide (E 551) since this material is not used as a food additive. However, their presence in the food additive cannot be excluded due to a lack of precision in the specifications for E 551.
- no carcinogenic effects were reported from chronic feeding studies at the highest doses tested of 7,500 mg silica gel/kg bw per day in mice and 2,500 mg silica gel/kg bw per day in rats,
- the material tested (silica gel, Syloid 244) in these chronic studies did not cover the full-size range of the nanoparticles that could be present in the food additive E 551 according to information provided by industry and the current EU specifications which contain no particle size limits,
- in the absence of a long-term study with nano silicon dioxide, the Panel was unable to extrapolate these results to a material complying with the current specifications for E 551, potentially containing nanoparticles,

the Panel concluded that:

- the EU specifications for silicon dioxide (E 551) are insufficient to adequately characterise silicon dioxide used as a food additive. They should include characterisation of particle size distribution using appropriate statistical descriptors (e.g. range, median, quartiles) as well as the percentage (in number and by mass) of particles in the nanoscale (with at least one dimension < 100 nm) present in silicon dioxide (E 551) used as a food additive. The measuring methodology applied should comply with the EFSA Guidance document (EFSA Scientific Committee, 2011a,b).
- from the available database there was no indication for toxicity of silicon dioxide (E 551) at the reported uses and use levels.
- due to the limitations in the available database described above the Panel was unable to confirm the current acceptable daily intake (ADI) 'not specified'.

The Panel considered that it would be possible to derive an ADI should the limitations in the toxicological database be reduced. The Panel noted that there were a number of approaches, which could decrease these limitations, which included but were not limited to a chronic toxicity study conducted according to a recognised guideline and with an adequately characterised material representative of SAS used as a food additive E 551.

The Panel recommended that:

- The European Commission considers lowering the current limits for toxic elements (arsenic, lead, mercury and cadmium) in the EU specifications for silicon dioxide (E 551) in order to ensure that the food additive will not be a significant source of exposure to these toxic elements in food.

The European Commission considers revising the current EU specifications for E 551 to include characterisation of particle size distribution using appropriate statistical descriptors (e.g. range, median, quartiles) as well as the percentage (in number and by mass) of particles in the nanoscale (with at least one dimension < 100 nm) present in silicon dioxide (E 551) used as a food additive. The measuring methodology applied should comply with the EFSA Guidance document (EFSA Scientific Committee, 2011a,b).

Table of contents

1. Introduction

The present opinion document deals with the re-evaluation of silicon dioxide (E 551) when used as a food additive.

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background as provided by the European Commission

Regulation (EC) No 1333/2008[1] of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under the Regulation (EU) No 257/2010[2]. This Regulation also foresees that food additives are re-evaluated whenever necessary in light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU of 2001. The report "Food additives in Europe 2000" submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with a highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of adoption of Regulation (EU) 257/2010 the 2003 Terms of References are replaced by those below.

1.1.2. Terms of Reference as provided by the European Commission

The Commission asks the EFSA to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

1.2. Information on existing evaluations and authorisations

Silicon dioxide (E 551) is authorised as a food additive in the EU in accordance with Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012[3].

In 1991, the SCF established a group acceptable daily intake (ADI) 'not specified' for sodium silicate (E 550), silicon dioxide (E 551), calcium silicate (E 552), magnesium silicate (E 553) and potassium silicate (E 560). The Committee argued that the available data confirmed the inertness of these compounds (SCF, 1991). No further details were given especially for silicon dioxide. Overall, the basis for the evaluation was not specified.

[1] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.

[2] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19–27.

[3] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1–295.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated silicon dioxide and a number of silicates in 1974. The committee established an ADI 'not limited' for silicon dioxide and certain silicates. The committee stated that the available data on orally administered silicon dioxide and silicates 'appear to substantiate the biological inertness of these compounds'. Furthermore, silicates absorbed from the gastrointestinal tract are excreted via urine and there is no evidence for toxic accumulation in the body (JECFA, 1974).

The Expert Group on Vitamins and Minerals (EVM) allocated a safe upper level (UL) of 25 mg/kg body weight (bw) per day (equivalent to 1,500 mg/day for a 60-kg adult) for human supplemental silica consumption over a lifetime (EVM, 2003). This allocation was based on a chronic feeding study in mice and rats (Takizawa et al., 1988) with amorphous silica (Syloid 244).

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) evaluated calcium silicate and silicon dioxide/silicic acid gel added for nutritional purposes to food supplements and concluded that the use of silicon dioxide up to 1,500 mg SiO_2/day added to food supplements is of no safety concern (EFSA ANS Panel, 2009). Silicon dioxide is included in the list of minerals which may be used in the manufacture of food supplements (Directive 2002/46/EC[4]).

In 2004, the EFSA Scientific Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) was asked to derive an UL for the intake of silicon from food that is unlikely to pose a risk of adverse health effects. The Panel stated that silicon occurs naturally in foods as silicon dioxide (silica) and silicates, and may also be added as an anticaking and antifoaming agent in the form of silica, silicates and dimethylpolysiloxane. The systemic availability of silicon from these sources varies, but is generally low. The Panel concluded that there were no suitable data for dose–response for establishment of an UL. However, the estimated dietary intake of 20–50 mg silicon/day corresponding to 0.3–0.8 mg/kg bw per day in a 60-kg person is unlikely to cause adverse effects (EFSA NDA Panel, 2004).

In 2009, the EFSA NDA Panel evaluated the scientific substantiation of health claims in relation to silicon, macrophage stimulation and an increase in circulating lymphocytes. The NDA Panel considered silicon to be sufficiently characterised but that the claimed effect 'immune health' was not sufficiently defined. The NDA Panel assumed that the claimed effect referred to aspects of 'stimulating macrophages' and 'increasing circulating lymphocytes'. The Panel considered that no evidence was provided that 'stimulating macrophages' and 'increasing circulating lymphocytes' were beneficial to the health of subjects with normal immune function and concluded that no cause and effect relationship was established between the consumption of silicon and 'stimulating macrophages' and 'increasing circulating lymphocytes' (EFSA NDA Panel, 2009.

In 2011, the EFSA NDA Panel evaluated the scientific substantiation of health claims in relation to the consumption of silicon and the protection against aluminium accumulation in the brain, cardiovascular health, the forming of a protective coat on the mucous membrane of the stomach, neutralisation of gastric acid, the contribution to normal formation of collagen and connective tissue, maintenance of normal bone, maintenance of normal joints, maintenance of a normal appearance and elasticity of the skin, and to the contribution to a normal formation of hair and nails. The NDA Panel concluded that, based on the data provided, a cause and effect relationship could not be established between the consumption of silicon and any of the above listed health claims (EFSA NDA Panel, 2011.

In 2014, the EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids (CEF Panel) delivered a statement on the safety of silanated silicon dioxide (FCM substance No 87) for use in food contact materials. The re-evaluation was requested because the substance has always been produced using synthetic amorphous silicon dioxide in the nanoform. Based on the available information, the CEF Panel considered that the absence of isolated primary nanoparticles in the basic silicon dioxide and in the silanated silicon dioxide to be adequately demonstrated, that the particle size range was not affected when the silanated product was incorporated into a low-density polyethylene film and that there was no detectable migration of silicon dioxide, of any particle size, from this film into appropriate food simulants. Therefore, the CEF Panel concluded that the substance did not raise a safety concern for the consumer in the currently authorised conditions of use (EFSA CEF Panel, 2014).

Silicon dioxide was reviewed by TemaNord (2002) along with calcium silicate, magnesium silicate, magnesium trisilicate and talc. TemaNord concluded that the toxicological data are less than would normally be required for these substances including an adequate carcinogenicity test.

[4] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Members States relating to food supplements. OJ L 183, 12.7.2002, p. 51.

In 2013, the European Commission's Joint Research Centre (JRC) published the characterisation of nano reference synthetic amorphous silica (SiO_2, SAS) (JRC depository materials: NM-200, NM-201, NM-202, NM-203, NM-204). The results for more than 15 endpoints were addressed, including physical–chemical properties, e.g. size and size distribution and crystallite size. Sample and test item preparation procedures were also described. The results were based on studies by European laboratories participating to the NANOGENOTOX Joint Action and by JRC (JRC, 2013).

Synthetic amorphous silicon dioxide (nano) (CAS No 112926-00-8) is authorised as an acting substance for use in biocidal products-type 18 (Commission implementing Regulation (EU) No 408/2014).[5]

Silicon dioxide (PM Ref. 86285, 86240) is included in the Union list of authorised substances that may be intentionally used in the manufacture of plastic layers in plastic materials and articles (Annex I to Commission Regulation (EU) No 10/2011[6]). Furthermore, silicon dioxide is permitted as an abrasive, absorbent, anticaking, bulking, opacifying and viscosity controlling agent in cosmetic products (European Commission database-CosIng[7]). Silicon dioxide is included in the European Union Register[8] of feed additives (Regulation (EC) No 1831/2003[9]).

In 2015, the EC Scientific Committee on Consumer Safety (SCCS, 2015) evaluated the safety of the nanomaterials silica, hydrated silica, silica silylate and silica dimethyl silylate for use in leave-on and rinse-off cosmetic (i.e. hair, skin, lip, face, nail) products. The SCCS concluded that the available data were inadequate and insufficient to allow any firm conclusions either for or against the safety of any of the individual SAS material, or any of the SAS categories that are intended for use in cosmetic products.

In 2016, the Food Standards Australia New Zealand (FSANZ, 2016) examined the scientific literature to determine whether there is robust evidence that adverse health effects may be associated with nano-forms (i.e. engineered nanomaterials (ENMs)) of insoluble inorganic food additives including amorphous silica (E 551). It was concluded that there was no evidence to suggest that there would be, at human dietary exposures, an unacceptable risk.

2. Data and methodologies

2.1. Data

The ANS Panel was not provided with a newly submitted dossier. EFSA launched public calls for data to collect information from interested parties.

The Panel based its assessment on information submitted to EFSA following the public calls for data, information from previous evaluations and additional available literature up to November 2017. Attempts were made at retrieving relevant original study reports on which previous evaluations or reviews were based; however, not always these were available to the Panel.

Food consumption data used to estimate the dietary exposure to silicon dioxide (E 551) were derived from the EFSA Comprehensive European Food Consumption Database (Comprehensive Database[10]).

The Mintel's Global New Products Database (GNPD) was used to verify the use of silicon dioxide (E 551) in food products. The Mintel's GNPD is an online database that contains the compulsory ingredient information present on the label of numerous products.

2.2. Methodologies

This opinion was formulated following the principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing guidance documents from the EFSA Scientific Committee.

[5] Commission implementing Regulation (EU) No 408/2014 of 23 April 2014 approving synthetic amorphous silicon dioxide as an existing active substance for use in biocidal products for product-type 18. OJ L 121, 24.4.2014, p. 17–19.
[6] Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. OJ L 12, 15.1.2011, p. 1–89.
[7] Available online: http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.simple
[8] Available online: http://ec.europa.eu/food/food/animalnutrition/feedadditives/comm_register_feed_additives_1831-03.pdf
[9] Regulation (EC) No 1831/2003 of the European Parliament and the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29–43.
[10] Available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

The ANS Panel assessed the safety of silicon dioxide (E 551) as a food additive in line with the principles laid down in Regulation (EU) 257/2010 and the relevant guidance documents: Guidance on submission for food additive evaluations by the SCF (2001).

When the test substance was administered in the feed or in the drinking water, but doses were not explicitly reported by the authors as mg/kg bw per day based on actual feed or water consumption, the daily intake was calculated by the Panel using the relevant default values as indicated in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012) for studies in rodents or, in the case of other animal species, by JECFA (2000a,b). In these cases, the daily intake is expressed as equivalent.

Dietary exposure to silicon dioxide (E 551) from its use as a food additive was estimated by combining food consumption data available within the EFSA Comprehensive Database with the maximum permitted levels and reported use levels submitted to EFSA following a call for data. The exposure was estimated according to different scenarios (see Section 3.4). Uncertainties in the exposure assessment were identified and discussed.

3. Assessment

In this opinion, the Panel did not consider data obtained with crystalline silica (an IARC class 1 carcinogen by inhalation (IARC, 1997)) because only the amorphous form of silicon dioxide is authorised as a food additive.

3.1. Technical data

3.1.1. Identity of the substance

According to Commission Regulation (EU) No 231/2012, the food additive silicon dioxide (E 551) is defined as 'an amorphous substance, which is produced synthetically by either a vapour-phase hydrolysis process, yielding **fumed silica**, or by a wet process, yielding **precipitated silica**, **silica gel** or **hydrous silica**. Fumed silica is produced in essentially an anhydrous state, whereas the wet-process products are obtained as hydrates or contain surface absorbed water'. The chemical formula is SiO_2 and the molecular weight is 60.08 g/mol (SiO_2). It is described as white, fluffy powder or granules; hygroscopic. The EINECS Number is 231-545-4.

According to Fruijtier-Poelloth (2012), 'there are three main types of silicon dioxide (silica, SiO_2) which are all found under the CAS No 7631-86-9, i.e. (i) crystalline silica, (ii) amorphous silica (naturally occurring or as a by-product in the form of fused silica or silica fume) and (iii) synthetic amorphous silica (SAS) including different forms: silica gel, precipitated silica, pyrogenic (fumed) silica and colloidal silica (silica sol)'.

The Panel noted that among these three types of silicon dioxide, SAS is the only one authorised as a food additive (E 551) and that SAS under the form of 'colloidal silica' is not authorised.

Further information was submitted by industry (CEFIC, 2017 (Documentation provided to EFSA n. 17)) on the forms of SAS used as a food additive E 551, which include pyrogenic (fumed) silica, and hydrated silica (precipitated silica, silica gel and hydrous silica) depending on the two process technologies (thermal process and wet process) used for their manufacture (Table 1).

Table 1: Overview of synthetic amorphous silica products used as a food additive (E 551) according to CEFIC (2016a, 2017 (Documentation provided to EFSA n. 15, 17))

Product	Route of production	Name in the EU specifications for E 551	EINECS no	CAS no, generic	CAS no, specific	Chemical abstract Index name
Pyrogenic silica	Thermal process	Fumed silica	231-545-4	7631-86-9	112945-52-5	Silica, amorphous, fumed; crystalline-free
Hydrated silica	Wet process	Precipitated silica	231-545-4	7631-86-9	112926-00-8	Synthetic amorphous silica, precipitated; crystalline-free
		Silica gel, hydrous silica	231-545-4	7631-86-9	112926-00-8	Synthetic amorphous silica, precipitated; crystalline-free

EINECS: European Inventory of Existing Commercial Chemical Substances; CAS: Chemical Abstracts Service.

According to industry (CEFIC, 2017 (Documentation provided to EFSA n. 17)), precipitated silica and silica gel are chemically identical but show some slight difference in physicochemical properties such as the pore size distribution (e.g. silica gel tends to have a narrower pore size distribution than precipitated silica). 'Hydrated' silica is synonym for the 'water-based production process' for precipitated silica and silica gel where the surface is covered by sylanol groups. Hydrous forms of silica gel are precursors of all silica gel products. Depending on the drying conditions and the content of water, the hydrous forms of silica gel are converted to: hydrous SAS with a loss of on drying described as 'not more than 70%'; SAS Xerogel with a loss of on drying described as 'not more than 8%'; SAS Aerogel that it is not used as a food additive.

According to industry (CEFIC, 2017 (Documentation provided to EFSA n. 17)), colloidal silica preparations are 'stabilized colloidal suspensions' of silica nanoparticles in liquids, usually water, and manufactured by different processes (ion exchange process with resins). The Panel noted that according to Regulation 231/2012, silicon dioxide (E 551) is described as a powder, whereas colloidal silica preparations are suspensions. However, the Panel was aware that stabilised colloidal silica preparations can be produced from fumed silica powder (Lim et al., 2010). The Panel further noted that those preparations of colloidal silica would then not fulfil the technological function of the food additive for the authorised uses. In addition, according to information from the industry 'colloidal silica is not sold as a food additive by ASASP member companies' (CEFIC, 2017, (Documentation provided to EFSA n. 17)).

Solubility:

ECETOC (2006) reported values for water solubility of synthetic amorphous silica at room temperature of 114–151 mg/L.

OECD SIDS reported solubility data on synthetic amorphous silica in water obtained from different studies:

- for pyrogenic silica: 15–24 mg/L (at 20°C, pH 5.6–6.6) and 36–68 mg/L (at 20°C, pH 5.5–5.8).
- for pyrogenic silica, precipitated silica and silica gel types under physiological conditions (water at 37°C, pH 7.1–7.4): 110–100 mg/L (i.e. 1.91 ± 0.05 to 2.76 ± 0.02 mmol/L).

Fruijtier-Poelloth (2012) reported for pyrogenic silica values of 144–151 mg/L and for silica gel, 127–141 mg/L in a simulated biological medium (at saturation, 37°C, pH 7.1–7.4).

According to CEFIC (2016a (Documentation provided to EFSA n. 15)), depending on the environmental conditions, SAS is either partially or completely soluble in water, and dissolves (depolymerises) in water generating orthosilicic acid (H_4SiO_4). At concentrations > 2 mmole/L, orthosilicic acid condenses with additional molecules of orthosilicic acid to form disilicic acid ($H_6Si_2O_7$), trisilicic acid, and oligo- and poly-silicic acids ($H_{2n+2}Si_nO_{3n+1}$).

According to CEFIC (2017 (Documentation provided to EFSA n. 17)), solubility is measured at equilibrium (in contrast to dissolution kinetics) and is difficult to be determined for 'silicon dioxide, amorphous'. The solubility is a function of the specific surface area of the material (measured by Brunauer–Emmett–Teller (BET) method and expressed as m^2/g). From the lowest to the highest BET range, a solubility range of 100–130 mg/L was found for precipitated silica and silica gel and from 110 to 250 mg/L for pyrogenic silica (OECD Test Guideline No 105; test duration 72–144 h at standard conditions without pH adjustment to reach the equilibrium). A similar solubility was reported in the study by Yang et al. (2016). However, the Panel noted that a solubility of 100–130 mg/L, equal to one part of substance dissolved in 10,000 parts of water, even when the equilibrium is reached, would classify the substance as 'very slightly soluble' according to the classification of the solubility by JECFA (2016).

According to JECFA specifications for silicon dioxide (JECFA, 2015), silicon dioxide is insoluble in water when solubility is determined after no more than 5 min (JECFA, 2016). According to industry (CEFIC, 2017 (Documentation provided to EFSA n. 17)), equilibrium cannot be achieved after such a short time in the case of pyrogenic or hydrated silica and for this reason there are differences in the solubility reported.

According to CEFIC (2016a (Documentation provided to EFSA n. 15)), the boiling point for silicon dioxide is 2,230°C.

Synonyms: silica, silicium dioxide (Commission Regulation (EU) No 231/2012).

An overview of the characteristics of the samples used in the biological and toxicological studies is given in Appendices A and B.

Particle size and particle size distribution of silicon dioxide (E 551)

According to information provided by interested parties (CEFIC, 2016b (Documentation provided to EFSA n. 16)), 'in order to exert its technological function as an anti-caking agent spacer, silicon dioxide (E 551) must have sizes larger than 100 nm'. Nanosized particles cannot exert this function. CEFIC further stated that: 'silicon dioxide (E 551) is characterised by primary structures ("primary particles"). These "primary particles" fuse to form aggregates which then, via hydrogen bonding, form agglomerates. Aggregates consist of a three-dimensional amorphous arrangement of covalently bound Si-O-Si with typically sizes > 100 nm. The aggregates withstand disaggregation. Even with high-energy processing "primary particles" are not liberated. Agglomerates can be separated into the original aggregates only by strong dilution and dispersion (e.g. in aqueous or organic solvents using stirrers and/or ultra-sonication). The mean diameter of synthetic amorphous silica is typically in the micrometre range'. CEFIC also stated that: 'this applies to all currently known SAS products in powder form'.

An overview of amorphous silica commercially available as a food additive E 551 and measured by different analytical techniques is given in Table 2 (CEFIC, 2016b, 2017, (Documentation provided to EFSA n. 16, 17)). The Panel noted that three different analytical methods were used (i.e. dynamic light scattering (DLS), laser diffraction (LD) and transmission electron microscopy (TEM)), that each method measures different particle characteristics and that this is reflected in the different numerical values obtained.

As regards the data obtained via DLS, interested parties (CEFIC, 2016b (Documentation provided to EFSA n. 16)) also provided data on the percentage of particles below 100 nm. However, it was stressed that to estimate the fraction below 100 nm for particle size distribution, multiple conversions need to be performed: i.e. intensity data must be converted to volume weighting, then volume weighting must be converted to number weighting. The interested parties stressed that these multiple conversions are feasible, however they lead to such uncertainties in the number weighting that these data are not reliable.

The Panel noted that in many studies reported in this opinion, the term 'nanomaterials' was often used to designate (structured) materials with sizes up to 1,000 nm (1 μm). These materials are, in fact, aggregates and/or agglomerates of primary particles generated during synthesis.

According to Commission Recommendation 2011/696/EU[11], 'nanomaterial' means 'a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm'.

The Panel considered materials to be 'nanosized' when they contain particles in a size range up to 100 nm.

The Panel considered that the food additive silicon dioxide (E 551) to be a nanosized material composed of aggregated 'primary particles'. The sizes of the aggregates and/or agglomerates are normally above 100 nm. However, depending on the material and/or on the manufacturing process, it cannot be excluded that some aggregates have a size below 100 nm.

According to Regulation (EU) 2015/2283[12] on novel foods, engineered nanomaterial means 'any intentionally produced material that has one or more dimensions of the order of 100 nm or less or that is composed of discrete functional parts, either internally or at the surface, many of which have one or more external dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic of the nanoscale'. The Panel noted that although engineered nano-SAS are not intended to be used as a food additive E 551, the current specifications would permit their use as a food additive E 551.

The Panel considered that several analytical methods are available to measure the particle size of nanomaterials, i.e. DLS, LD, TEM, scanning electron microscopy (SEM). As already stated, each of these methods measures different particle characteristics, which is reflected in the different numerical size-values obtained (Table 2).

As regards silicon dioxide, given the high surface reactivity of the particle, the primary particles spontaneously aggregate and agglomerate giving rise mainly to structures with sizes > 100 nm. When suspended in a liquid, SiO_2 particles adsorb solvent molecules, giving rise to structures with sizes up to the micrometre range. These facts were taken into consideration by the Panel when evaluating the safety of silicon dioxide (E 551) used as a food additive.

[11] Commission Recommendation of 18 October 2011 on the definition of nanomaterial. OJ L 275, 20.10.2011, p. 38–40.

[12] Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. OJ L 327, 11.12.2015, p. 1–22.

Table 2: Particle characteristics of commercially available synthetic amorphous silica (E 551) and used in the biological and toxicological studies. Data provided by interested parties (CEFIC, 2016b, 2017 (Documentation provided to EFSA n. 16, 17))

Type of material	Sample	Dynamic light scattering (DLS) intensity-weighted distribution (nm)					Laser diffraction (LD) volume-weighted distribution (μm)				Transmission electron microscopy (TEM) number-weighted distribution (nm)				
		Sample preparation	D_{10}	Mean[a]	D_{90}	Fraction (%) < 100 nm on number-based distribution	Sample preparation	D_{10}	Mean	D_{90}	Sample preparation	Feret diameter D_{10} (min–max)	Feret diameter mean (min–max)	Feret diameter D_{90} (min–max)	Fraction (%) < 100 nm on number-based distribution
Fumed silica	A	Ultrasonication 7 min; (Misonix XL2020 at amplitude 9). Sample: solution in H_2O at pH 10.5	76	138	162	70	Dry powder with a Microtrac air blender	20	99	205	Sample dispersed in ethanol	32–51	73–120	120–197	80–42
	B		95	164	193	39		20	90	183		35–56	101–168	178–297	55–27
	A						Disp. under mild sheer: 0.6%SAS deionized water	37	96	166					
	B							35.1	80	134					
	A						Disp. under mild sheer: 0.6%SAS deionized water	24.5	48	76					
	B							17	29	43					
	C	1 wt%, 100 mL H_2O. Ultrasonic., 3 min; 110 W	108.6	166.2	286.8	38.9	Dry powder dispersing system	430.9	681.7	965.7	Ultrasonication	43–74.9	141–240	270–464.6	41–19
	D	Disp. 0.3% in H_2O, ultrasonic energy input 50%; 3 min	100	175	360		Dry powder dispersed in air		20–50						
		Disp. 0.3% in H_2O, ultrasonic energy input 100%; 3 min	90	156.2	350										
	E	Disp. 0.3% in H_2O, ultrasonic energy input 50%; 3 min	110	186.8	370		Dry powder dispersed in air		20–50		Ultrasonic bath (120 w, 30 KHz), 3 min		183.5[b]		
		Disp. 0.3% in H_2O, ultrasonic energy input 100%; 3 min	90	169.1	396										

Type of material	Sample	Dynamic light scattering (DLS) intensity-weighted distribution (nm)					Laser diffraction (LD) volume-weighted distribution (μm)				Transmission electron microscopy (TEM) number-weighted distribution (nm)				
		Sample preparation	D_{10}	Mean[(a)]	D_{90}	Fraction (%) < 100 nm on number-based distribution	Sample preparation	D_{10}	Mean	D_{90}	Sample preparation	Feret diameter D_{10} (min–max)	Feret diameter mean (min–max)	Feret diameter D_{90} (min–max)	Fraction (%) < 100 nm on number-based distribution
Precipitated silica	F	Disp. 0.3% in H_2O, ultrasonic energy input 50%; 3 min	107	1,975	160	Not given	60 s sonification in water with dispersion aid		100–140		Not given				
		Disp. 0.3% in H_2O, ultrasonic energy input 100%; 3 min	120	699.8	950										
		Disp. 0.3% in H_2O, ultrasonic energy input 100%; 6 min	115	311	4,160										
	G	Disp. 0.3% in H_2O, ultrasonic energy input 50%; 3 min	150	2,787	1,800		60 s sonification in water with dispersion aid		35–65		Not given				
		Disp. 0.3% in H_2O, ultrasonic energy input 100%; 3 min	160	831.3	4,800										
	H	Disp. 0.3% in H_2O, ultrasonic energy input 50%; 3 min	270	622.1	2,000		60 s sonification in water with dispersion aid		3–6		Not given				
		Disp. 0.3% in H_2O, ultrasonic energy input 100%; 3 min	240	474.5	1,000										
	I	Concentration 1% w/v. Magnetic stirring 15 min	Not given	Not given	Not given						Concentration 1% w/v. Magnetic stirring 15 min	D_{min}/ aggregate 27	D_{min}/ aggregate 169	D_{min}/ aggregate 353	55
		Concentration 0.8% w/v. Ultrasonication 3 min	290.6	498.0	5,426.1	46.7	Not given				Concentration 0.8% w/v. Ultrasonication 3 min	D_{min}/ aggregate 24	D_{min}/ aggregate 108	D_{min}/ aggregate 251	65
		Concentration 0.8% w/v. Ultraturrax 15 min	149.5	848.0	3,100.9	0.0	Not given				Concentration 0.8% w/v. Ultraturrax 15 min	D_{min}/ aggregate 20	D_{min}/ aggregate 90	D_{min} of aggregate 225	77

Type of material	Sample	Dynamic light scattering (DLS) intensity-weighted distribution (nm)					Laser diffraction (LD) volume-weighted distribution (μm)				Transmission electron microscopy (TEM) number-weighted distribution (nm)				
		Sample preparation	D_{10}	Mean[(a)]	D_{90}	Fraction (%) < 100 nm on number-based distribution	Sample preparation	D_{10}	Mean	D_{90}	Sample preparation	Feret diameter D_{10} (min–max)	Feret diameter mean (min–max)	Feret diameter D_{90} (min–max)	Fraction (%) < 100 nm on number-based distribution
Silica gel	J	1 wt%, 100 mL H_2O. Ultrasonic, 3 min 110 W		643.7	0	3.3	Not given				Not given				

(a): Cumulant algorithm in nm.
(b): Average minimum Feret diameter.

Information on the particles size of silicon dioxide (E 551) as reported in the literature is presented in Appendix C.

3.1.2. Specifications

The specifications for silicon dioxide (E 551) as defined in the Commission Regulation (EU) No 231/2012 and by JECFA (2015) are listed in Table 3.

Table 3: Specifications for silicon dioxide (E 551) according to Commission Regulation (EU) No 231/2012 and JECFA (2015)

	Commission Regulation (EU) No 231/2012	**JECFA (2015)**
Definition	Silicon dioxide is an amorphous substance, which is produced synthetically by either a vapour-phase hydrolysis process, yielding fumed silica, or by a wet process, yielding precipitated silica, silica gel, or hydrous silica. Fumed silica is produced in essentially an anhydrous state, whereas the wet-process products are obtained as hydrates or contain surface absorbed water	Silicon dioxide is an amorphous substance, which is produced synthetically by either a vapour-phase hydrolysis process, yielding pyrogenic (fumed) silica, or by a wet process, yielding precipitated silica (silica gel). Pyrogenic silica is produced in an anhydrous state, whereas the wet process products are obtained as hydrates or contain surface absorbed water (information required on hydrated silica, silica aerogel and colloidal silica)
Assay	Content after ignition not less than 99.0% (fumed silica) or 94.0% (hydrated forms)	Pyrogenic (fumed) silica: Not less than 99% of SiO_2 on the ignited basis Precipitated silica (silica gel): Not less than 94% of SiO_2 on the ignited basis Hydrated silica: Information required Silica aerogel: Information required Colloidal silica: Information required
Description	White, fluffy powder or granules. Hygroscopic	Pyrogenic silica: a pyrogenic silicon dioxide occurring as a fine, white amorphous power or granules Precipitated silica (silica gel): a precipitated, hydrated silicon dioxide occurring as a fine, white, amorphous powder, or as beads or granules Hydrated silica: *Information required* Silica aerogel: *Information required* Colloidal silica: *Information required*
Identification		
Test for silica	Positive	Passes test
Solubility	–	Insoluble in water and insoluble in ethanol (Information required)
Purity		
Loss on drying	Not more than 2.5% (fumed silica, 105°C, 2 h) Not more than 8.0% (precipitated silica and silica gel, 105°C, 2 h) Not more than 70% (hydrous silica, 105°C, 2 h)	Pyrogenic silica: Not more than 2.5% (105°, 2 h) Precipitated silica (silica gel): Not more than 8% (105°, 2 h) Hydrated silica: Information required Silica aerogel: Information required Colloidal silica: Information required
Loss on ignition	Not more than 2.5% after drying (1,000°C, fumed silica) Not more than 8.5% after drying (1,000°C, hydrated forms)	Pyrogenic silica: Not more than 2.5% (1,000°, 1 h) on dried sample Precipitated silica, silica gel and hydrated silica: Not more than 8.5% (1,000°, 1 h) on dried sample Hydrated silica: *Information required* Silica aerogel: *Information required* Colloidal silica: *Information required*
Soluble ionisable salts	Not more than 5.0% (as Na_2SO_4)	–
Arsenic	Not more than 3 mg/kg	Not more than 3 mg/kg *(information required)*
Lead	Not more than 5 mg/kg	Not more than 5 mg/kg (information required)
Mercury	Not more than 1 mg/kg	–

The Panel noted that, according to the EU specifications for silicon dioxide (E 551), impurities of the toxic elements arsenic, lead and mercury are accepted up to concentrations of 3, 5 and 1 mg/kg, respectively. Contamination at these levels could have a significant impact on exposure to these toxic elements, which are already close to the health based guidance values or benchmark doses (lower confidence limits) established by EFSA (EFSA CONTAM Panel, 2009, 2010, 2012, 2014).

The Panel noted that there are no limits for the particle size of silicon dioxide (E 551) in the EU specifications (Commission Regulation (EU) No 231/2012).

The Panel is aware that the specifications for 'silicon dioxide, amorphous' were discussed at the 84th JECFA meeting (JECFA, 2017).

3.1.3. Manufacturing process

Two different process technologies are used for the manufacture of SAS, (i) the thermal process resulting in the production of pyrogenic or fumed silica and (ii) the wet process yielding precipitated silica, silica gel or hydrous silica.

- **Thermal process**

  In the information provided by interested parties (CEFIC, 2016b, 2017 (Documentation provided to EFSA n. 15, 17)), the production process of pyrogenic (fumed) SAS was summarised as being produced by hydrolysis of volatile chlorosilanes (e.g. tetrachlorosilane) in an oxygen (air)/hydrogen gas flame reactor.

$$2H_2 + O_2 \rightarrow 2H_2O$$

$$SiCl_4 + 2H_2O \rightarrow SiO_2 + 4HCl$$

  The pyrogenic (fumed) silica forms agglomerates inside the cooling system. The solid particles are separated from the off-gas (contains hydrochloric acid), e.g. by filtering. Afterwards, additional adsorbed hydrochloric acid on the surface of the silica is removed by a de-acidification step. The product is then bagged, filled into containers, or loaded into silo cars.
  The reaction parameters are kept under strict control to achieve uniform product quality. No raw materials of animal or plant origin and no organic solvents are used to manufacture pyrogenic SAS.
  According to data from ELC (2009 (Documentation provided to EFSA n. 26)), $SiCl_4$ is converted, in the reactor, to the gaseous phase and reacts completely in a flame (flame temperature > 1,000°C) with the intermediately formed water to form SiO_2. It is stated that the size of the SiO_2 particles in the reactor are in the range of 5–50 nm which, along the temperature gradient in the reactor, grow into larger aggregates of about 100 nm and then form agglomerates with sizes of 1–250 μm.

- **Wet process**

  Precipitated silica

  According to CEFIC (2017 (Documentation provided to EFSA n. 17)), precipitated amorphous silica is manufactured by the precipitation of diluted aqueous alkali metal silicate (e.g. waterglass[13] solution, $Na_2O.xSiO_2$, x = 2–4) with a diluted acid (e.g. sulfuric acid or hydrochloric acid) in water according to following reaction:

$$nNa_2O{\cdot}xSiO_2 + nH_2SO_4 \rightarrow nNa_2SO_4 + xSiO_2 + nH_2O$$

  The solid content of the precipitate is typically between 50 and 200 g/L. The precipitate is then filtered, washed to remove salts, dehydrated and milled. After drying the precipitated silica can be milled to achieve the specified particle size distribution.
  After a period of time (up to 2 h), a gelatinous precipitate is formed. The particle size is about 500–600 μm (ECETOC, 2006; ELC, 2009 (Documentation provided to EFSA n. 26)).

[13] Waterglass: sodium salt of silicic acid that forms silicic acid upon acidification (Napierska et al., 2010).

Silica gel

According to information provided by interested parties (CEFIC, 2016a, 2017 (Documentation provided to EFSA n. 15, 17)), silica gels are produced by the neutralisation of diluted aqueous alkali metal silicates, e.g. waterglass, with a diluted acid (e.g. sulfuric acid) according to following reaction.

$$nNa_2O \cdot xSiO_2 + nH_2SO_4 \rightarrow nNa_2SO_4 + xSiO_2 + nH_2O$$

The first step comprises the formation of a hydrosol, produced by the controlled mixing of the sodium silicate solution (waterglass; $Na_2O.xSiO_2$, x = 2–4) and diluted mineral acid (usually sulfuric acid, but other acids may also be used). The transformation of the solution into the gel state is characterised by an increase in viscosity and the development of an internal structure with larger aggregates until the complete material reaches a solid state. By controlling the washing, ageing and drying conditions, the functional physical parameters (i.e. porosity, pore size and particle size distributions, degree of aggregation and/or agglomeration, surface areas) are adjusted to produce a range of different silica gel products. Side products such as sodium sulfate are removed in the washing step. After drying, silica gels are milled to achieve the specified particle size distribution.

If the pH is reduced to below pH 7 or if salt is added, the chemical subunits tend to fuse together in chains resulting in the formation a gel structure (silica gel). If the pH is kept neutral or alkaline (pH 7–10), then the subunits stay separated, and they gradually grow. These products are called silica sols (Iler, 1979).

3.1.4. Methods of analysis in food

Analysis of silicon (Si)

An overview of methods for analysis of silicon in environmental and biological media is given in ECETOC (2006). The most common methods for sensitive silicon analysis are inductively coupled plasma atomic absorption spectrometry (ICP-AES) and flameless atomic absorption spectrometry (AAS) (Carlisle, 1997).

The Association of Official Agricultural Chemists (AOAC) published a gravimetric method for determination of sand and silica in plant material (AOAC, 2000). Test items are burned and alkali-soluble silicon dioxide is dissolved in sodium hydroxide. After filtration, silicon is precipitated with HCl, dried and weighed.

JECFA (2015) included a method for the determination of silicon based on ICP-AES. The sample is burned together with potassium hydroxide and boric acid, dissolved in water, washed and analysed spectrometrically. This method is also applicable for measurements in biological media (ECETOC, 2006).

Analysis of silicon dioxide particles in food products

The Panel noted that in some studies reported in this section, the term 'nanomaterials' was often used to designate (structured) materials with sizes above 100 nm. However, these materials are, in fact, aggregates and/or agglomerates of primary particles, and not primary particles per se.

The Panel noted that, according to information provided by industry, the size of primary particles for SAS used as a food additive E 551 is in the range of 5–15 nm (as measured by TEM) (Appendix A).

Dekkers et al. (2011) reported the presence of silica particles (50–200 nm) in 12 food products containing E 551. The authors used hydrodynamic chromatography with inductively coupled plasma mass spectrometry (HDC-ICP-MS) to determine the particle size and determine the concentration of silicon and derive the silica content in the selected foods. The percentage of nano-silica particles (in mass compared to total silica) was found to be between 4% (in a steak rub) and up to 33% (in an instant asparagus soup).

Chun Yin Lee (2013) in a comprehensive study analysed the physicochemical characteristics of silica nanoparticles in complex food matrices. Field flow fractionation-inductively coupled plasma mass spectrometry (FFF-ICP-MS) separation and detection technique was used to analyse synthetic amorphous silica (E 551) in coffee creamers. The FFF-ICP-MS system was connected to a multiangle light scattering (MALS) detector to enable the measurement of the silica nanoparticle sizes. The authors concluded that the choice of FFF parameters as well as sample preparation play an important and influential role in the separation of silica nanoparticles from its complex matrix.

Contado et al. (2013) analysed an instant barley coffee (stated to be silica free) and an instant cappuccino (containing E 551) both in powder form. The samples were obtained, along with a food integrator (not further specified, but stated to be rich in E 551) from a local grocery. Size characterisation was performed using sedimentation field flow fractionation (SdFFF), SEM, TEM and photon correlation spectroscopy (PCS). The authors stated that the synergic use of these analytical techniques made it possible, for some samples, to confirm the presence of primary nanoparticles (10 nm) organised in clusters or aggregates of different dimension and, for others, to discover that the information obtained via these techniques was incomplete, particularly as regards the presence of small particles. The authors concluded that most of the silica particles were organised in aggregates or agglomerates of sizes larger than 100 nm; the food integrator showed a more heterogeneous population of aggregates than the cappuccino mixture. The cappuccino, on the other hand, presented only a limited number of isolated particles smaller than 100 nm. In their conclusion, the authors also emphasised that particular care must be taken in all analysis steps because SiO_2 particle integrities are sensitive to the media (pH, ionic strength, surfactant type), and dispersive procedures.

Heroult et al. (2014) used FFF-ICP-MS connected with MALS and TEM for the analysis of silicon and nano-silica in a coffee creamer and in a commercial coffee creamer containing the food additive silicon dioxide (E 551). Gentle sonication in a water bath was applied to facilitate the particle distribution. Different FFF carriers were tested to evaluate their impact in the particle aggregate formation. The nano-silica fraction detected by FFF-ICP-MS was approximately 11% of the total silicon measured in coffee creamer.

Athinarayanan et al. (2015) reported the results of the characterisation by TEM of SiO_2 from two different food products (a commercial brand of 'zero calorie' sweetener and a commercial brand of a powdered vanilla flavour) and a commercial SiO_2, (E 551). The TEM images of E 551 from the food products or commercial E 551 showed that the food additive used in food consisted of particles with a primary particle size of 20–50 nm. The particles were aggregated; the analysis by DLS showed an average particle size of SiO_2 of 160 nm.

Barahona et al. (2016) described a multimethod approach for the detection and characterisation of food-grade synthetic amorphous silica nanoparticles. Eleven different food-grade samples were analysed using DLS, MALS, asymmetric flow-field flow fractionation (AF4), inductively coupled plasma mass spectrometry (ICP-MS) and TEM. It was shown that, in general, the z-average, AF4 hydrodynamic diameters and root mean square radii were in good agreement. AF4-ICP-MS coupling and prechannel calibration with nano-silica standards allowed the reliable detection of nanoparticles below 100 nm for 10 of 11 samples (AF4 diameters between 20.6 and 39.8 nm) and the mass quantification in seven different samples (at mg/L concentrations). TEM characterisation included the determination of the minimum detectable size and subsequent measurement of the equivalent circle diameter of primary particles and small aggregates, which were between 10.3 and 20.3 nm. The authors stressed that because the dynamic size application range was limited by the minimum detectable size (i.e. size $>$ 1 nm), the techniques used in the study could only be used as positive tests (i.e. demonstration of the presence of particles with sizes $>$ 1 nm).

Contada et al. (2016) used, in addition to the analytical techniques as in the study described above Contado et al. (2013), also differential centrifugal sedimentation (DCS). The techniques were used to analyse powders of instant barley coffee (silica free) and of instant cappuccino mix (containing E 551) also obtained from an Italian local grocery. The food products were prepared as indicated on their labels for a homemade preparation. From the analytical data obtained, the authors concluded that the two commercial food products when dispersed in water (as suggested on their labels in terms of stirring and concentration), contained nanoparticles only when analysed by DCS. Barley coffee did not contain silica particles, while the cappuccino mix contained silica (as additive E 551) in a concentration well below 1% w/w.

Yang et al. (2016) characterised six food-grade SiO_2 (fumed, pyrogenic, white powders not labelled as 'nano', commercially available in the US), on their morphology (by TEM), hydrodynamic diameter and zeta (ζ) potential (by PALS), crystal structure (by X-ray diffraction (XRD)) and surface functionality (by X-ray photoelectron spectroscopy (XPS)). Samples were described as 'pristine' SiO_2 because they had not been mixed or reacted with food matrices. All samples contained agglomerates of SiO_2. The mean diameter of all primary particles (TEM analysis) were $<$ 100 nm with mean primary particle sizes in a range of 9 $\pm$ 6 to 26 $\pm$ 8 nm. The hydrodynamic diameters of the agglomerates in samples were in the range of 1,223 $\pm$ 468 to 1,579 $\pm$ 88 nm. The authors stressed that these sizes are much larger than the primary diameters measured by TEM, because SiO_2 agglomerates when suspended in water. The authors also quantified the occurrence and examined the structural characteristics of SiO_2 present in 14 products (in foods, anticaking agents and in personal care products available on the US market, no further specification). Based on XRD,

XPS and TEM analyses, it was shown that SiO_2 in the samples was of the same morphology and size as the 'pristine' bulk food-grade SiO_2 and exhibited consistent morphologies ranging in size from below 100 to > 500 nm. The extraction method from food was, however, not described.

3.1.5. Stability of the substance, and reaction and fate in food

According to information provided by interested parties (CEFIC, 2016a (Documentation provided to EFSA n. 15)), amorphous silicon dioxide is an inert substance that has a tendency to adsorb moisture and volatile substances. The shelf life is between 12 and 36 months from the date of production.

Furthermore, it was stated that silicon dioxide is a rather inert substance and that no degradation products under normal conditions are known (Holleman, 2007; EFSA ANS Panel, 2009).

3.2. Authorised uses and use levels

Maximum levels of silicon dioxide (E 551) have been defined in Annex II to Regulation (EC) No 1333/2008[14] on food additives, as amended. In this document, these levels are named maximum permitted levels (MPLs).

Currently, silicon dioxide (E 551) is an authorised food additive in the EU in 22 food categories as listed in Table 4. Several food categories are authorised at MPLs ranging from 2,000 to 30,000 mg/kg and others at *quantum satis* (QS). Silicon dioxide (E 551) can be authorised together with silicates (E 552, E 553a and E 553b).

Table 4 summarises foods that are permitted to contain silicon dioxide (E 551) and the corresponding MPLs as set by Annex II to Regulation (EC) No 1333/2008.

Table 4: MPLs of silicon dioxide (E 551) in foods according to the Annex II to Regulation (EC) No 1333/2008

Food category number	Food category name	E-number/ group	Name	Restrictions/ exceptions	MPL (mg/L or mg/kg as appropriate)
0	Food additives permitted in all categories of foods	E 551–553	Silicon dioxide – silicates	Only foods in dried powdered form (i.e. foods dried during the production process, and mixtures thereof), excluding foods listed in table 1 of Part A of Annex II	10,000
0	Food additives permitted in all categories of foods	E 551–553	Silicon dioxide – silicates	Only foods in tablet and coated tablet form, excluding the foods listed in table 1 of Part A of Annex II	QS
01.7.2	Ripened cheese	E 551–553	Silicon dioxide – silicates	Only sliced or grated cheese hard and semi-hard cheese	10,000
01.7.5	Processed cheese	E 551–553	Silicon dioxide – silicates		10,000
01.7.6	Cheese products (excluding products falling in category 16)	E 551–553	Silicon dioxide – silicates	Only sliced or grated hard and semi-hard products	10,000
01.8	Dairy analogues, including beverage whiteners	E 551–553	Silicon dioxide – silicates	Only sliced or grated cheese analogues and processed cheese analogue; beverage whiteners	10,000
02.2.2		E 551–553			30,000

[14] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16.

Food category number	Food category name	E-number/ group	Name	Restrictions/ exceptions	MPL (mg/L or mg/kg as appropriate)
	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions		Silicon dioxide – silicates	Only tin greasing products	
02.3	Vegetable oil pan spray	E 551–553	Silicon dioxide – silicates	Only tin greasing products	30,000
05.2	Other confectionery including breath refreshening microsweets	E 551–553	Silicon dioxide – silicates	Surface treatment only	QS
05.3	Chewing gum	E 551	Silicon dioxide	Surface treatment only	QS
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	E 551–553	Silicon dioxide – silicates	Surface treatment only	QS
11.1	Sugars and syrups as defined by Directive 2001/111/EC	E 551–553	Silicon dioxide – silicates	Only dried powdered foods	10,000
11.1	Sugars and syrups as defined by Directive 2001/111/EC	E 551–553	Silicon dioxide – silicates	Only foods in tablet and coated tablet form	QS
11.4.2	Table Top Sweeteners in powder form	E 551–553	Silicon dioxide – silicates		10,000
11.4.3	Table Top Sweeteners in tablets	E 551–553	Silicon dioxide – silicates		QS
12.1.1	Salt	E 551–553	Silicon dioxide – silicates		10,000
12.1.2	Salt substitutes	E 551–553	Silicon dioxide – silicates		20,000
12.2.2	Seasonings and condiments	E 551–553	Silicon dioxide – silicates	Only seasoning	30,000
13.1.3	Processed cereal-based foods and baby foods for infants and young children as defined by Directive 2006/125/EC	E 551	Silicon dioxide	Only dry cereals	2,000
17.1[(a)]	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	E 551–553	Silicon dioxide – silicates		QS
17.2[(a)]	Food supplements supplied in a liquid form	E 551–553	Silicon dioxide – silicates		QS
17.3[(a)]	Food supplements supplied in a syrup-type or chewable form	E 551–553	Silicon dioxide – silicates		QS

MPL: maximum permitted level; QS: *quantum satis*.
(a): FCS 17 refers to food supplements as defined in Directive 2002/46/EC of the European Parliament and of the Council excluding food supplements for infants and young children.

According to Annex III, Part 1, silicon dioxide (E 551) is authorised as a carrier in emulsifiers and colours at QS.

According to Annex III, Part 2 of Regulation (EC) No 1333/2008, silicon dioxide (E 551) is also authorised as a food additive other than carrier in foods additives, in dry powdered colour preparations

and dry powdered preparations of emulsifiers at the maximum level of 50,000 mg/kg in the preparation and in E 508 potassium chloride and E 412 guar gum preparations and in dry powder preparations of polyols at the maximum level of 10,000 mg/kg in the preparation.

Added to that, according to Annex III, Part 2 of Regulation (EC) No 1333/2008, silicon dioxide (E 551) is also authorised as a food additive other than carrier in foods additives, in E 1209 polyvinyl alcohol-polyethylene glycol-*graft*-co-polymer at the maximum level of 5,000 mg/kg in the preparation; in dry powdered extracts of rosemary (E 392) at the maximum level of 30,000 mg/kg in the preparation; in potassium nitrate (E 252) at the maximum level of 10,000 mg/kg in the preparation.

According to Annex III, Part 3, silicon dioxide (E 551) is authorised as a food additive including carriers in food enzymes, at the maximum level of 50,000 mg/kg in the dry powdered enzyme preparation with a maximum level in final food and beverages at QS.

According to Annex III, Part 4, silicon dioxide (E 551) is authorised as a food additive including carriers in food flavourings, at the maximum level of 50,000 mg/kg in all flavourings.

In addition, according to Annex III, Part 5, Section A of Regulation (EC) No 1333/2008, silicon dioxide (E 551) and calcium silicate (E 552) are also authorised as food additives in nutrients except nutrients intended to be used in foods for infant and young children listed in point 13.1 of Part E of Annex II, in dry powdered preparations of all nutrients at the maximum level of 50,000 mg/kg in the dry powdered preparation (singly or in combination) and silicon dioxide (E 551) is authorised in potassium chloride preparations used in salt substitutes at the maximum level of 50,000 mg/kg in the preparation.

Finally, according to Annex III, Part 5, Section B of Regulation (EC) No 1333/2008, silicon dioxide (E 551) is also authorised as a food additive added in nutrients intended to be used in foods for infants and young children in dry powdered nutrient preparations at the maximum level of 10,000 mg/kg.

3.3. Exposure data

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, information on actual use levels is required for performing a more realistic exposure assessment, especially for those food additives for which no MPL is set and which are authorised according to QS.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued a public call[15] for occurrence data (usage level and/or analytical data) on silicon dioxide (E 551). In response to this call, updated information on the actual use levels of silicon dioxide (E 551) in foods was made available to EFSA by industry. No analytical data on the concentration of silicon dioxide (E 551) in foods were made available by the Member States.

3.3.1. Summarised data on reported use levels in foods provided by industry

Industry provided EFSA with data on use levels (n = 520) of silicon dioxide (E 551) in foods. Out of these, 375 use levels were reported on 19 out of the 22 food categories in which silicon dioxide (E 551) is authorised according to Annex II to Regulation (EC) No 1333/2008 (Table 4). No data were submitted for the food categories FC 02.2.2 (Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions), 02.3 (Vegetable oil spray) and 12.1.2 (salt substitutes). The other 145 reported use levels related to foods in which silicon dioxide (E 551) could be present due to its authorisation in FC 0 or as carry-over.

Updated information on the actual use levels of silicon dioxide (E 551) in foods was made available to EFSA by Food Chemical Risk Analysis (FCRA), European Dairy Association (EDA), KRÜGER GmbH & Co., Food Drink Europe (FDE), International Chewing Gum Association (ICGA), Grace Materials Technologies, Dr Loges Naturheilkunde neu entdecken, Specialised Nutrition Europe (SNE), European Federation of Associations of Health Products Manufacturers (EHPM), Association of the European Self-Medication Industry (AESGP) and Food Supplements Europe (FSE).

In total, 92 usage levels on silicon dioxide (E 551) referred to niche products. These usage levels were reported mainly on food supplements and flavoured drinks. They were excluded from the exposure assessment when other usage levels were available for these food categories.

Many data were provided on the food as sold, before reconstitution. For instance, food in powder to which liquid should be added before consumption (instant coffee, dehydrated soups, etc.). The

[15] Available online: http://www.efsa.europa.eu/sites/default/files/consultation/151012.pdf

reported use levels were derived applying the dilution factors indicated by data providers or the same factor for similar foods when a dilution factor was not available.

Appendix D provides data on the use levels of silicon dioxide (E 551) in foods as reported by industry.

3.3.2. Summarised data extracted from the Mintel's Global New Products Database

The Mintel's GNPD is an online database which monitors new introductions of packaged goods in the market worldwide. It contains information of over 2 million food and beverage products of which more than 900,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 20 out of its 28 member countries and Norway presented in the Mintel's GNPD.[16]

For the purpose of this Scientific Opinion, the Mintel's GNPD[17] was used for checking the labelling of food and beverage products and food supplements for silicon dioxide (E 551) within the EU's food market, between January 2012 and July 2017, as the database contains the compulsory ingredient information on the label.

According to the Mintel's GNPD, silicon dioxide (E 551) was labelled on almost 5,000 products, of which half were food supplements. Food products labelled with silicon dioxide (E 551) belonged mainly to the following food subcategories of the Mintel's GNPD: Instant Noodles, Creamers, Malt & Other Hot Beverages, and Meal Replacements & Other Drinks. The percentages of foods labelled to contain silicon dioxide (E 551) ranged from less than 0.1% in many food subcategories to 24.5% in the Mintel's GNPD food subcategory 'Vitamins & Dietary Supplements'; the overall average percentage was 1.0%.

Appendix E lists the percentage of the food products labelled with silicon dioxide (E 551) out of the total number of food products per food subcategory according to the Mintel's GNPD food classification.

3.3.3. Food consumption data used for exposure assessment

EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a). New consumption surveys added to the Comprehensive database in 2015 were also taken into account in this assessment.[10]

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category (FC) and the level of detail used for exposure calculations, uncertainties could be introduced owing to possible subjects' underreporting and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the currently best available source of food consumption data across Europe.

Food consumption data from the following population groups were used in the exposure assessment: infants, toddlers, children, adolescents, adults and the elderly. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 5).

[16] Missing Bulgaria, Cyprus, Estonia, Latvia, Lithuania, Luxembourg, Malta and Slovenia.
[17] Available online: http://www.gnpd.com/sinatra/home/ accessed on 28/7/2017.

Table 5: Population groups considered for the exposure estimates of silicon dioxide (E 551)

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From more than 12 weeks up to and including 11 months of age	Bulgaria, Denmark, Finland, Germany, Italy, UK
Toddlers[(a)]	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, Netherlands, Spain, UK
Children[(b)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, UK
The elderly[(b)]	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Netherlands, Sweden, UK

(a): 'Toddlers' in the EFSA Comprehensive Database corresponds to 'young children' in Regulations (EC) No 1333/2008 and (EU) No 609/2013.
(b): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from the FoodEx classification system has been linked to the food categorisation system (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D, to perform exposure estimates. In practice, the FoodEx food codes were matched to the FCS food categories.

Food categories considered for the exposure assessment of silicon dioxide (E 551)

The food categories in which the use of silicon dioxide (E 551) is authorised were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system), at the most detailed level possible (up to FoodEx Level 4) (EFSA, 2011b).

Some food categories or their restrictions/exceptions are not referenced in the EFSA Comprehensive Database and could therefore not be taken into account in the present estimate. This was the case for four food categories and may have resulted in an underestimation of the exposure. The food categories which were not taken into account are (in ascending order of the FCS codes):

- 01.7.6 Cheese products, only sliced or grated hard and semi-hard products;
- 02.2.2 Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions, only tin greasing products;
- 02.3 Vegetable oil pan spray, only tin greasing products;
- 12.1.2 Salt substitutes.

For the following food categories, the restrictions/exceptions which apply to the use of silicon dioxide (E 551) were also not referenced. As restrictions represent a large part of the food category, the whole food category was considered in the exposure assessment. This applied to five food categories and may have resulted in an overestimation of the exposure:

- 01.7.2 Ripened cheese, only sliced or grated cheese hard and semi-hard cheese. The full food category was taken into account because the restriction represents a large part of the whole food category.
- 01.8 Dairy analogues, including beverages whiteners, only sliced or grated cheese analogues and processed cheese analogue; beverages whiteners. The full food category was taken into account because the restriction represents a large part of the whole food category.

The following two sugars and syrups categories represent most of foods under FC 11.1 thus whole food category was included in the assessment:

- 11.1 Sugars and syrups as defined by Directive 2001/111/EC, only dried powdered foods
- 11.1 Sugars and syrups as defined by Directive 2001/111/EC, only foods in tablet and coated tablet form
- 13.1.3 Processed cereal-based foods and baby foods for infants and young children as defined by Directive 2006/125/EC, only dry cereals. Considering that this is a sensitive population and that infants usually eat the same foods (brand-loyalty), all foods classified under this food category were included in the assessment.

It has to be noted that silicon dioxide (E 551) is authorised in FC 0, meaning in 'all categories of foods excluding foods for infants and young children, except where specifically provided for'. In the case of silicon dioxide (E 551), two restrictions apply to this food category:

- only foods in dried powdered form (i.e. foods dried during the production process, and mixtures thereof), excluding foods listed in Table 1 of Part A of Annex II,
- only foods in tablet and coated tablet form, excluding the foods listed in Table 1 of Part A of Annex II.

For this reason, in addition to food categories listed in Table 4, other food categories for which data were submitted and referring to foods sold in dried powdered form (to be reconstituted before consumption) or in (coated) tablet form were also considered in the current exposure assessment. This concerns the following food categories:

- 01. Dairy products,
- 04.2.6 Processed potato products,
- 08.2 Meat preparations: for meat burger,
- 10.2 Processed eggs,
- 11.2 Other sugars and syrups,
- 12.2.1 Herbs and spices,
- 12.5 Soups and broths,
- 12.6 Sauces,
- 14.1.4 Flavoured drinks (which can come from powder mainly cocoa beverages; drinks such as cola were excluded),
- 14.1.5 Coffee or instant tea,
- 16 Desserts.

Silicon dioxide is also authorised in FC 12.1.1 salts and FC 12.2.2 seasonings and condiments and few uses were reported for these usages in foods but not all food products containing added salts were taken into account.

Data were also submitted for the FCs 13.2, 13.3 and 18 in which silicon dioxide (E 551) is not authorised as such but could be present as for FC 0 or from carry-over. However, considering that FC 18 is very unspecific, the foods belonging to this food category (e.g. processed foods and prepared or composite dishes) were reclassified under food categories in accordance to their main ingredient and included as such in the exposure assessment. Also, the food items belonging to FCs 13.2 and 13.3, consumed by the population groups children, adolescents, adults and the elderly, may be very diverse; in addition, there was very limited information on their consumption. Therefore, eating occasions belonging to these FCs were also reclassified under food categories in accordance with their main component. The use levels of silicon dioxide (E 551) available for FCs 13.2, 13.3 and 18 were not considered in the exposure assessment.

In addition, the restrictions which apply to the use of silicon dioxide (E 551) for the FCs 17.1, 17.2 and FC 17.3 (Food supplements, in solid, liquid and syrup-type or chewable form) could not be taken into account, and therefore, the whole food category (FC 17) was considered in the specific exposure scenario including food supplements (Section 3.4.3).

Additional food categories for which use levels were submitted were also taken into account in the exposure estimates considering the presence of silicon dioxide (E 551) due to carry-over (Annex III of Regulation No 1333/2008) as reported by industry. This relates to the following food categories: flavoured fermented milk products including heat-treated products (FC 01.4), edible ices (FC 03), cocoa and chocolate products as covered by Directive 2000/36/EC (FC 05.1), fillings of stuffed pasta (FC 06.4.5), fine bakery wares (FC 07.2), meat products (FC 08.3), soups and broths (FC 12.5), sauces (FC 12.6), dietary foods for infants for special medical purpose and special formulae for infants (FC 13.1.5.1), and potato-, cereal-, flour- or starch-based snacks (FC 15.1). All use levels used in the exposure assessment are listed in Appendix F.

Out of the 22 food categories in which silicon dioxide is authorised according to annex II, 15 were considered in both the maximum and refined exposure scenarios. Additionally, 18 food categories were considered because of being powdered foods (via FC 0) or due to the presence of silicon dioxide (E 551) due to carry-over.

3.4. Exposure estimates to silicon dioxide (E 551) from its use as a food additive

The Panel estimated the chronic dietary exposure to silicon dioxide (E 551) for the following population groups: infants, toddlers, children, adolescents, adults and the elderly. Dietary exposure to silicon dioxide (E 551) was calculated by multiplying concentrations of silicon dioxide (E 551) per food category (Appendix F) with their respective consumption amount per kilogram of body weight for each individual in the EFSA Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period. Dietary surveys with only one day per subject were excluded as they are considered as not adequate to assess repeated exposure.

This was carried out for all individuals per survey and per population group, resulting in distributions of individual exposure per survey and population group (Table 5). Based on these distributions, the mean and 95th percentile of exposure were calculated per survey and per population group. The 95th percentile of exposure was only calculated for those population groups with a sufficiently large sample size to allow this calculation (EFSA, 2011a). Therefore, in the present assessment, the 95th percentiles of exposure for infants from Italy and for toddlers from Belgium, Italy and Spain were not estimated.

Exposure assessment to silicon dioxide (E 551) was carried out by the ANS Panel based on: (1) MPLs as set down in the EU legislation and maximum levels of data reported to EFSA (defined as the *regulatory maximum level exposure assessment scenario*); and (2) reported use levels (defined as the *refined exposure assessment scenario*). These two scenarios are discussed in detail below.

These scenarios did not consider the exposure to silicon dioxide (E 551) via the intake of food supplements or consumption of foods for special medical purposes (FSMP). The exposure via the intake of food supplements was covered in an additional exposure scenario described below. Only one use level was reported by industry on a niche product of FSMP described as special infant formulae (Appendix D). A specific exposure scenario covering consumers of FSMP was not performed.

3.4.1. Regulatory maximum level exposure assessment scenario

The regulatory maximum level exposure assessment scenario is based on the MPLs as set in Annex II to Regulation (EC) No 1333/2008 and listed in Table 4 and/or on the maximum reported use levels provided by industry for food categories in which the food additive is allowed at QS, as described in the EFSA Conceptual framework (EFSA ANS Panel, 2014). In the case of QS, only those QS food categories can be considered in this scenario for which use levels were submitted.

The Panel considers the exposure estimates derived following this scenario as the most conservative as it is assumed that the population groups will be exposed to silicon dioxide (E 551) present in food at the MPL and maximum reported use levels over a longer period of time.

3.4.2. Refined exposure assessment scenario

The refined exposure assessment scenario is based on use levels reported by industry. This exposure scenario can consider only food categories for which these data were available to the Panel.

Appendix F summarises the use levels of silicon dioxide (E 551) used in the refined exposure assessment scenario. Based on the available data set, the Panel calculated two refined exposure estimates based on two model populations:

- The brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to silicon dioxide (E 551) present at the maximum reported use level for one food category. This exposure estimate is calculated as follows:
  - Combining food consumption with the maximum of the reported use level for the main contributing food category at the individual level.
  - Using the mean of the typical reported use levels for the remaining food categories.

- The non-brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to silicon dioxide (E 551) present at the mean reported use level in food. This exposure estimate is calculated using the mean of the typical reported use levels for all food categories.

3.4.3. 'Food supplement consumers only' scenario

Silicon dioxide (E 551) is authorised in FC 17 Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children. As exposure via food supplements may deviate largely from that via food, and the number of food supplement consumers may be low depending on populations and surveys, the exposure to silicon dioxide (E 551) was calculated according to an additional scenario in order to reflect additional exposure to food additives from the intake of food supplements.

This scenario was estimated assuming that consumers of food supplements were exposed to silicon dioxide (E 551) present at the maximum reported usage levels in food supplements. For the remaining food categories, the mean of the typical reported use levels was used.

As FC 17 does not consider food supplements for infants and toddlers as defined in the legislation, exposure to silicon dioxide (E 551) from food supplements was not estimated for these two population groups.

Appendix F summarises the use levels of silicon dioxide (E 551) used in this specific exposure assessment scenario.

3.4.4. Dietary exposure to silicon dioxide (E 551)

Table 6 summarises the estimated exposure to silicon dioxide (E 551) from its use as a food additive in six population groups (Table 5) according to the different exposure scenarios. Detailed results per population group and survey are presented in Appendix H.

Table 6: Summary of dietary exposure to silicon dioxide (E 551) from their use as a food additives in the *regulatory maximum level exposure assessment scenario* and in the refined exposure scenarios, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day)

	Infants (12 weeks–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Regulatory maximum level exposure assessment scenario						
Mean	18.5–74.2	18.5–39.4	10.2–31.2	7.0–18.5	4.9–13.2	3.9–10.1
95th percentile	43.6–162.7	38.2–71.9	25.0–79.2	16.3–36.0	10.6–29.9	8.4–23.7
Refined estimated exposure assessment scenario						
Brand-loyal scenario						
Mean	2.8–11.0	9.0–18.9	5.9–24.5	4.1–9.9	2.8–8.0	2.3–6.5
95th percentile	10.6–26.4	16.9–44.6	14.7–61.0	9.6–21.3	5.6–18.8	4.3–16.7
Non-brand-loyal scenario						
Mean	0.8–5.3	3.0–7.4	2.7–18.4	1.7–4.1	0.9–2.7	0.7–2.6
95th percentile	3.4–13.6	7.7–14.9	5.6–49.7	3.9–8.9	2.3–6.4	1.7–5.6

In all exposure scenarios, the lowest exposure was reported in the elderly while the highest was in infants at the MPL scenario and in children for the refined one. In the *regulatory maximum level exposure assessment scenario*, the mean and high (95th percentile) exposure to silicon dioxide (E 551) from its use as a food additive ranged from 3.9 to 74.2 mg/kg bw per day and from 8.4 to 162.7 mg/kg bw per day, respectively. The corresponding estimates of exposure in the *refined estimated exposure brand-loyal scenario* were 2.3 and 24.5 mg/kg bw per day for the mean exposure and 4.3 and 61 mg/kg bw per day for the high exposure. In the *non-brand-loyal scenario*, the mean and high exposure to silicon dioxide (E 551) from its use as a food additive ranged from 0.7 to 18.4 mg/kg bw per day and from 1.7 to 49.7 mg/kg bw per day, respectively.

In the food supplements, consumers only scenario, across the four populations of children, adolescents, adults and the elderly, mean and high exposure to silicon dioxide (E 551) from its use as a food additive ranged from 3.1 to 25 mg/kg bw per day and from 7.3 to 34 mg/kg bw per day, respectively.

The main food categories contributing to the exposure to silicon dioxide (E 551) are presented in Appendix D. In all scenarios and population groups, the main contributors were FC 1.7.2 'ripened cheese', FC 11.1 'sugars and syrups as defined by Directive 2001/111/EC' and FC 7.2 'fine bakery wares' (except in infants).

3.4.5. Uncertainty analysis

Uncertainties in the exposure assessment of silicon dioxide (E 551) have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and summarised in Table 7.

Table 7: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction[(a)]
Consumption data: different methodologies/representativeness/underreporting/misreporting/ no portion size standard	+/–
Use of data from food consumption surveys covering only a few days to estimate high percentiles (95th percentile) long-term (chronic) exposure	+
Correspondence of reported use levels to the food items in the EFSA Comprehensive Database: uncertainties to which types of food the levels refer	+/–
Uncertainty in possible national differences in use levels within food categories	+/–
Concentration data: • use levels considered applicable to all foods within the entire food category, whereas on average 1% of the foods, belonging to food categories with foods labelled with additive, was labelled with the additive	+
Food categories selected for the exposure assessment: exclusion of food categories due to missing FoodEx linkage (n=4/22 food categories authorised according to Annex II)	–
Food categories selected for the exposure assessment: inclusion of food categories without considering the restriction/exception (n = 5/22 food categories authorised according to Annex II)	+
Food categories included in the exposure assessment: • Only foods belonging to FC 0 for which data were available were included in the exposure estimates	–
Regulatory maximum level exposure assessment scenario: • exposure calculations based on MPLs according to Annex II to Regulation (EC) No 1333/2008 and maximum reported use levels (reported use from industries) • foods which may contain the food additive only according to Annex III to Regulation (EC) No 1333/2008 partly taken into account	+ –
Refined exposure assessment scenarios: • exposure calculations based on the maximum or mean levels (reported use from industry) • foods which may contain the food additive only according to Annex III to Regulation (EC) No 1333/2008 only partly taken into account	+ –

(a): +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

The food categories contributing most to the exposure in the different population groups were FC 1.7.2 'ripened cheese', FC 11.1 'sugars and syrups as defined by Directive 2001/111/EC' and FC 7.2 'fine bakery wares' (except in infants) (Section 3.4.4). The Panel noted that the information retrieved from the Mintel's GNPD showed that the percentage of foods belonging to the most comparable food subcategories according to the Mintel's GNPD classification (e.g. 'Hard Cheeses & Semi-Hard Cheeses' 'Cakes, Pastries & Sweet Goods') was less than 1%. For FC 11.1 'sugars and syrups as defined by

Directive 2001/111/EC', no comparable food subcategory could be identified. This percentage demonstrates that the general assumption that all foods belonging to a food category contain the food additive has likely resulted in an overestimation of the exposure to silicon dioxide (E 551) in all exposure scenarios.

The Panel considered that the uncertainties identified would result in an overestimation of the exposure to silicon dioxide (E 551) in the refined exposure scenario at the reported uses and used levels.

3.5. Exposure estimate to silicon dioxide (E 551) from uses other than as a food additive

Silicon dioxide is also used in cosmetic products, as excipient in drugs and as a source of silicon in food supplements (Directive 2002/46/EC). Silicon is an inorganic compound, which is broadly present in the natural environment. Quantification of exposure via all these sources is not precisely known and could therefore not be taken into account in this opinion. The Panel noted that it was reported (Vance et al., 2015) that exposure of consumers to silicon dioxide nanoparticles containing products was mostly by dermal route then by inhalation; consumption of foods being a lower contributor.

3.6. Biological and Toxicological data

Not having a full biological and toxicological database for each of the different forms of silicon dioxide authorised as a food additive, the Panel has considered the available information from different SAS items for the hazard identification. The Panel was aware that the SAS test items used in the biological studies available were very different in the form of silicon dioxide and chemically modified silicon dioxide and in particle size distribution due to the various manufacturing processes and starting materials used. In addition, a number of studies were available with chemically modified amorphous silicon dioxide particles such as some of those used by the pharmaceutical industry. These studies were not included in the present assessment as this material was clearly different from silicon dioxide (E 551) used as a food additive.

The Panel noted that because of their specific physicochemical properties, engineered nanoparticles of SAS are not representative of silicon dioxide used as a food additive (E 551). However, because nanoparticles of silicon dioxide are present in the food additive E 551 (see Table 2), studies performed with specifically designed engineered nanoparticles of SAS have also been included in this assessment in order to assess any toxicity associated with nanoparticles present in the food additive, provided they were prepared using amorphous silicon dioxide. Toxicity studies performed with chemically modified SAS nanoparticles of SAS, were not considered in the present opinion.

The Panel noted that in some studies (especially those conducted in the 1960-1970s) while the authors reported analysis of 'silica' content, analytical methods available at the time were only capable of measuring silicon. The Panel considered that while this was expressed as silica by the authors, it was not possible to determine whether it was silica or silicon that was measured.

3.6.1. Absorption, distribution, metabolism and excretion (ADME)

Studies with SAS

In vivo studies

Rats

The oral administration (no further details) of an aqueous suspension of precipitated silica (FK700; Appendix A) to rats (strain not specified) at a dose of 1,500 mg/kg bw per day for 1 month did not result in accumulation of silica. The average silica content of the liver (1.5 μg), kidney (6.4 μg) and spleen (5.3 μg) was not significantly different from control values of 1.8, 7.2 and 7.8 μg silica, respectively (Degussa AG, 1968, as referred to by ECETOC, 2006).

When rats (strain not specified) received for 20 days an oral dose of 100 mg (about 500 mg/kg bw per day) of fumed silica (HDK V15; Appendix A), the silica contents in liver and kidney but not in spleen were slightly increased compared to controls: 4.2 μg in liver of treated rats vs 1.8 μg in controls, spleen 5.5 μg vs 7.2 μg in controls and kidney 14.2 μg vs 7.8 μg in controls (Klosterkötter, 1969; as referred to by ECETOC, 2006). The Panel noted that in this study, contrary to what was reported for liver and kidney, the silica content in the spleen did not increase. Due to the non-availability of the full report, the Panel could not assess the biological significance of this observation.

In the van der Zande et al. (2014) study, Sprague–Dawley rats (groups of five male rats) were fed a diet containing either fumed SAS (commercially available E 551, with a primary particle size of 7 nm) or nano-fumed silica (NM-202; JRC, 2013; Appendix B). The animals received 0, 100, 1,000 or 2,500 mg/kg bw per day of fumed SAS, or 100, 500 or 1,000 mg/kg bw per day of NM-202. Additional groups of animals were exposed for 84 days to 2,500 mg/kg bw per day SAS or 1,000 mg/kg bw per day NM-202. While exposure to SAS or NM-202 did not result in clearly elevated tissue 'silica' concentration after 28 days of exposure, after 84 days 'silica' accumulation was reported in the spleen of animals exposed to SAS, but not to NM-202. The Panel noted that only the total silica content could be determined in tissues because of the use of ICP-MS. The authors concluded that: 'Additional studies seem warranted to further evaluate the biological relevance of the possible accumulation of silica in the spleen of SAS exposed animals'. The Panel agreed with the authors (for additional discussion about the reliability of this study see Section 3.6.3).

Guinea pigs

'Guinea pigs were fed with SAS (precipitated, sol) mixed in the diet, or administered diluted SAS directly, or by intraperitoneal injection. There was no significant difference in total and dissolved silica levels excreted in urine (analysed colorimetrically). The concentration of silica in tissues (liver, kidney, lung, heart, muscle) was determined by the silicomolybdic acid reaction. The absolute level was low in all tissues (maximum 12.63 mg/100 g dry matter in lungs). The silica concentration in tissues was apparently not influenced by the silica concentration in the diet. Experiments with radio-labelled 31SiO2 indicated that orally administered silica sol was rapidly absorbed and excreted. The prolonged ingestion of a SAS-containing diet did not result in any storage of silica' (Sauer et al., 1959a,b; as referred to by ECETOC, 2006).

Human studies

Studies with precipitated silica (FK 700; Appendix A) and fumed silica (AEROSIL, crystalline-free (particle size from 10 to 40 nm) were performed in volunteers (each group comprised five men and one woman; aged 22–28 years, not on a controlled diet). Suspensions of the test item in apple juice were given in the morning and midday. Each dose contained 1,250 mg of the test substance. The total urine was collected for 3 days pre-application (control values) and for 4 days post-application. Silicon in urine was determined after alkaline hydrolysis; other excretion routes were not evaluated. During the 4 days post-treatment, no significant changes of the renal silicon excretion were noted compared with pre-application values. For both test substances, less than 0.5% of the applied silica was excreted via urine. For fumed silica, the individual control values of the pretest phase ranged from 25 to 87 mg/day. In the post-treatment phase, individual mean excretion rates ranged from 32 to 61 mg/day. For precipitated silica, individual mean excretion rates ranged from 16 to 71 mg/day in the pretest phase and from 20 to 81 mg/day in the post-treatment. The authors concluded that the changes in silicon excretions were within the range of normal physiological variation, and there was little indication of absorption of silica after ingestion (Degussa AG, 1966 (Documentation provided to EFSA n. 18); Lang, 1966 (Documentation provided to EFSA n. 50); Langendorf and Lang, 1967). The Panel noted that the administered dose was very high and that excretion in faeces was not measured.

Silica gel (Syloid HC; Appendix A) was administered for 3 weeks with the morning and evening meal to six human adults (3 men, 3 women; aged 20–51 years) with primary type II hyperlipoproteinaemia (Grace, 1982, as referred to by ECETOC, 2006). The starting dose of 1,000 mg/day was daily increased by 1,000 mg/day, up to a final dose of 16,000 mg/day. The test item was not absorbed significantly from the intestine (no further information including analytical methods).

Studies with intentionally engineered nano-SAS

In vitro studies

Yoshida et al. (2014) studied *in vitro* the intestinal absorption of amorphous (nano- and micro-) silica particles with diameters of 70, 300 and 1,000 nm (nSP70, mSP300 and mSP1000, respectively) and of nSP70 that had been surface-modified with carboxyl or amine groups (nSP70-C and nSP70-N, respectively). Analysis of intestinal absorption by using the everted gut sac method combined with an inductively coupled plasma optical emission spectrometer showed that the intestinal absorption of nSP70-C and NSP-N was significantly greater than that of nSP70, mSP300 and mSP1000, which were poorly absorbed. According to the authors, these results indicated that silica nanoparticles can be absorbed through the intestine and that particle diameter and surface properties are major

determinants of the degree of absorption. According to the authors, interactions between proteins and nanomaterials play important roles in the biological effects and bio-distribution of nanomaterials.

Zane et al. (2015) observed that after incubation at a concentration of 100 mg/cm^2, stable fluorophore/silica nanoparticles (30 nm) were visible in the cytoplasm but not in the nucleus of cells from the mouse macrophage cell line MH-S, and of C2BBe1 (derived from Caco-2) cells.

Lee et al. (2017) studied the intestinal transport mechanisms of silicon dioxide (E 551) (primary particles 27 nm (SEM) purchased from Evonik) and bulk silicon dioxide (primary particles 4,000 nm (SEM), purchased from ABC Nanotech Co) using an *in vitro* culture model of human intestinal follicle-associated epithelium (FAE). The effect of the presence of food components, such as sugar and protein, on the absorption of nanoparticles was also evaluated by measuring silicon urinary excretion. The results demonstrated that absorption of nanoparticles (3.94 ± 0.38%) was greater than that of bulk materials (2.95 ± 0.37%), possibly due to intestinal transport by microfold (M) cells.

Animal studies

So et al. (2008) reported an oral repeated-dose toxicity study with silica nanoparticles (30–90 nm, obtained from rice husk, not further specified). Groups of Balb/c and C57BL/6 mice (five males and five females per group) were fed either normal diet (control), 1% silica nanoparticles diet (equivalent to 2,000 mg/kg bw per day) or 1% microsized silica (0.5–30 μm) diet for 10 weeks. The silicon content was measured in lung and liver by ICP-AES. The exposure resulted in higher serum alanine aminotransferase (ALT) levels in mice dosed with silica nanoparticles compared to mice dosed with microsized silica and the control mice. The study is not well reported and lacks some important details on, among others, the characterisation of the silica nanoparticles both in the feed and in the organs. It is also remarkable that the increased ALT was only found for Balb/c mice and not for C57BL/6 mice. Finally, the Panel noted that, as already noted by others (Fruijtier-Poelloth, 2012), the test material was amorphous silica of biogenic origin and may include impurities of crystalline silica.

In the study by Cho et al. (2009), a fluorescent dye-labelled silica particle suspension of 50, 100 and 200 nm sizes was intravenously injected to groups of BALB/c mice (n = 5 males) to identify their tissue distribution and excretion. The authors claimed that silica particles of 50, 100 and 200 nm were cleared via urine and bile. Silica particles, identified by the fluorescent dye, were trapped by macrophages in the spleen and liver until 4 weeks after the single injection. Excretion to urine and faeces showed different patterns depending on particles size. At 12 h, 50 nm nanoparticles reached the highest concentration in urine, and 100 nm particles had a peak concentration at 24 h. All three sized silica particles were detected in urine only 1 week after injection. Silica particles eliminated slower via faeces than in urine. The 200 nm silica particles were excreted from urine and faeces at lower concentrations than 50 and 100 nm particles. The Panel noted that the study has a number of limitations; in particular the authors measured the fluorescent dye assuming that the dye was still connected with the particles but they did not confirm this assumption, and the number of animals per group (n = 5) was low.

He et al. (2011) investigated the bio-distribution and excretion of mesoporous silica particles and polyethylene glycol-treated mesoporous silica particles (PEG–MSNs) of different particle sizes (80, 120, 200 and 360 nm) in groups of male ICR mice (n = 5) and Sprague–Dawley rats (n = 3) injected with 20 mg/kg bw of the suspensions via the tail-vein. Mesoporous silica particles and PEG–MSNs of different particle sizes were mainly localised to the liver and spleen, a minor proportion was localised to the lung, and a few particles were found in the kidney and heart. The biodistribution percentages of MSNs and PEG–MSNs of the particle sizes of 80 and 120 nm in liver and spleen firstly decreased, then increased, and finally decreased again; however, those of 200 and 360 nm decreased continuously in the time period from 30 min to 1 month after injection. According to the authors, PEGylation reduced mesoporous silica particles localisation to the liver, spleen and lung, and resulted in longer blood circulation lifetimes, slower biodegradation and correspondingly lower amounts of degradation products of PEG–MSNs than mesoporous silica particles of the same particle sizes. Mesoporous silica particles of smaller particle sizes had reduced localisation to the liver and spleen tissues, and were more slowly biodegraded and correspondingly had a lower excreted amount of degradation products.

Fu et al. (2013) investigated the absorption, distribution and excretion of 50 mg/kg bw silica nanoparticles with an average size of 110 nm after intravenous, subcutaneous, intramuscular injection and oral administration to female ICR mice. The excretion and distribution of silica nanoparticles were achieved by quantitatively assessing the silicon content in the liver, spleen, kidney, lung, muscle, intestine, intestine content, faeces and urine 24 h and 7 days after administration using inductively coupled plasma-optical emission spectrometry (ICP-OES). Furthermore, fluorescein isothiocyanate doped silica nanoparticles nanocomposites were designed to track the *in vivo* distribution of silica

nanoparticles. TEM was carried out to characterise the distribution of silica nanoparticles in the liver, spleen and intestine. A fraction of the silica nanoparticles administered by intramuscular and sub-cutaneous injections crossed biological barriers into the liver but with a low absorption rate. After oral administration, silica nanoparticles were absorbed and localised in the liver, whereas silica nanoparticles administered by intravenous injection were mainly localised in the liver and spleen. Silica nanoparticles were mainly excreted via the urine and faeces irrespective of the route of administration.

In the NANOGENOTOX project (online), nano-fumed silica (NM-200 and NM-203; JRC, 2013; Appendix B) were used. Sprague–Dawley rats were administered orally by gavage (with 20 mg/kg bw once or for five consecutive days), which results in a total administration of 100 mg/kg bw. After oral administration, a very limited increase in the silicon content was observed in the spleen but mainly in the liver in animals receiving five consecutive administrations by 2 days after administration. This was transient and silicon content in all organs was almost back to the background level on day 14 after administration. When SiO_2 nanoparticles were administered intravenously with the same total dose, silicon content in organs was much higher than after oral dosing, with silicon mainly in the liver where it persisted up to 90 days after administration.

Yun et al. (2015) examined the systemic toxicity (see Section 3.6.3, for full description of the study) of silica nanoparticles with a primary particle size of 12 nm (TEM) by oral administration to Sprague–Dawley rats (12/sex and per group). In a study performed according to the OECD Test Guideline 408, the animals were orally administered doses of 0, 245, 490 or 980 mg/kg bw per day for 90 days. The authors reported that silica was not systemically distributed in tissues and that most of the ingested silica was excreted in the faeces.

Zane et al. (2015) used stable fluorophore/silica nanoparticles (around 30 nm) with surface characteristics similar to those of commercial silica particles. The particles were administered to mice Charles River females) (sonicated for 15 s in water suspension before administration; 1 mg/mouse for 4 consecutive days) by gavage. The animals were killed 3 h after the final administration, and the presence of fluorophore was analysed in various organs, including the stomach, small intestine, caecum, colon, kidney, lung, brain and spleen. The Panel noted that the authors did not use TEM to demonstrate the presence of nanoparticles in the tissues; however, the authors concluded that, by combining confocal fluorescence microscopy with ICP-MS, they could demonstrate the presence of nanoparticles, rather than their dissolved form, was established mainly in liver tissues.

Interactions of silica nanoparticles with the food matrix, biological milieu and gastrointestinal tract (GIT) parameters

In vitro

Peters et al. (2012) used an *in vitro* model mimicking the different stages of human digestion (mouth, gastric, intestinal) to study the presence, dissolution, agglomeration and release of material in the nanosize range from various foods (black coffee, powdered soup and pancake and hot water) and containing silicon dioxide (E 551) (primary particles 7 nm) or with added SAS or with engineered silica nanoparticles. Results are expressed as a mass percentage of nanosized silica (5–200 nm) relative to the total amount of silica in or added to the food item. In the mouth stage of digestion, particles with a size range of 5–50 nm and 50–500 nm were present in food products with E 551, added SAS or added engineered silica nanoparticles. During the gastric stage, nanosized silica was no longer present in the food matrices of coffee and instant soup, while small amounts were found in pancake. According to the authors, the absence of nanoparticles of silica in the gastric stage could be due to the low pH combined with high electrolyte concentration. Under these conditions, DLS and SEM examination revealed that large silica agglomerates were formed. In the subsequent intestinal digestion stage, the nanosized silica particles reappeared again.

Tenzer et al. (2013) used label-free snapshot proteomics to obtain quantitative time-resolved profiles of human plasma coronas formed on silica nanoparticles of various size and surface functionalisation. Complex time- and nanoparticle-specific coronas, which comprise almost 300 different proteins, were found to form rapidly (< 0.5 min) and, over time, to change significantly in terms of the amount of bound protein. According to the authors, corona formation affected haemolysis, thrombocyte activation, nanoparticle uptake and endothelial cell death.

Sakai-Kato et al. (2014) studied the absorption on human Caco-2 cells of amorphous silica particles (diameters of 50, 100 or 200 nm) in fasted- and fed-state simulated gastric or intestinal fluids. The sizes and the intracellular transport of the particles into Caco-2 cells and Caco-2 monolayer membrane permeability were evaluated. When the silica particles were dispersed in fasted- and fed-state

simulated gastric fluids and fasted-state simulated intestinal fluids, the mean of the three types of particle sizes were not affected. In contrast, silica particles of 50, 100 or 200 nm dispersed in fed-state simulated intestinal fluids agglomerated (size > 1,000 nm). The intracellular amounts of silica particles (all sizes) dispersed in fasted-state simulated gastric fluids were similar to controls cultured in medium. However, when the different particles were dispersed in fed-state simulated intestinal fluids, the amounts of intracellular particles significantly decreased compared to control. Similar results were reported on the transcellular transport of silica particles. The authors concluded that food matrix had an effect on the agglomeration of silica particles and, furthermore, that the larger the size of silica particles, the lower the absorption into and/or transport through the cells.

A series of *in vitro* studies with fumed and precipitated silica (AEROSIL 380F and SIPERNAT 22S; Appendix A) were available to the Panel (Maier et al., 2013, 2014, 2015 (Documentation provided to EFSA n. 55, 56 and 57); only abstract available). Maier et al. (2013, 2014 (Documentation provided to EFSA n. 55 and 56) reported that *in vitro*, pyrogenic and precipitated silica in a tomato soup did not present significant changes in both their structure and size distribution after heating in water or an acidic medium simulating the gastric environment (pH 1.3) as well as in a fed-state simulated intestinal fluid (pH 5). In a consecutive report, no degradation or structural change was observed when a mixture of the same material with potato starch or saccharose was exposed to simulated gastrointestinal conditions (Maier et al., 2015 (Documentation provided to EFSA n. 57)). The Panel noted that these data provided some evidence that pyrogenic and precipitated silica appears to be stable under these *in vitro* conditions but because it was poster presentations, the description of the studies was limited.

In vivo

Lee et al. (2017) studied the solubility, absorption, tissue distribution and excretion kinetics of silicon dioxide (E 551) (primary particles 27 nm (SEM)) and bulk silicon dioxide (primary particles 4,000 nm) following single-dose oral administration to rats. The effect of the presence of food components, such as sugar and protein, on the absorption of nanoparticles was also evaluated by measuring silicon urinary excretion. Particle size was found to have no significant effect on *in vivo* dissolution, biodistribution or excretion kinetics. The absorption profile of silica nanoparticles was highly dependent on the presence of sugar or protein, showing an accelerated absorption rate in the presence of glucose, presumably due to a surface interaction on nanoparticles. The authors concluded that interactions between nanoparticles and food components should be considered when evaluating biological impacts and toxicity.

Mc Clements et al. (2017) reviewed the potential effects of food components on the behaviour, in the GIT, of various engineered nanoparticles including silicon dioxide. The authors highlighted some important physicochemical and colloidal mechanisms by which the food matrix (foods and their digestion products) may impact the gastrointestinal fate of inorganic nanoparticles. For instance, the authors noted that: 'Foods vary widely in their compositions, structures, and physical properties, and this can lead to broad alterations on the physicochemical characteristics of the nanoparticles, which can thus influence their release, transport, solubility, aggregation state, surface chemistry, corona formation, and absorption'. The Panel considered that this should be considered in the interpretation of the biological data.

Overall, the Panel agreed with the conclusion of the review by Bellmann et al. (2015) that '*in vitro* and *in silico* fluid incubation data provided some evidence of changes in particle stability, aggregation, and surface properties following interaction with luminal factors present in the GI tract. The variables included physical forces, osmotic concentration, pH, digestive enzymes, other food, and endogenous biochemicals, and commensal microbes'. The Panel also agreed with the statement that 'knowledge of the most influential luminal parameters will be essential when developing models of the GI tract to quantify the per cent absorption of food-relevant engineered NMs for risk assessment'.

The Panel also noted that in biological fluids, proteins bind to the surface of nanoparticles in general (Grunér et al., 2016), and of nanoparticles of silica in particular (Lesniak et al., 2012; Docter et al., 2014; Mirshafiee et al., 2016; Kurtz-Chalot et al., 2017; Strojan et al., 2017), to form a coating known as the protein corona, which can critically affect the interaction of any nanoparticles with living systems. Monopoli et al. (2012) reported that unless they are specifically designed to avoid it, nanoparticles in contact with biological fluids are rapidly covered by a selected group of biomolecules to form a corona, which may be linked to their biological impacts. The composition of this corona may have consequence on the biological reactivity of the particles as, for instance, it has been reported that some proteins of the complement system, which have significant roles in the development of inflammation, are present in the corona after incubation of silicon dioxide nanoparticles in serum (Strojan et al., 2017).

Other ADME studies

Van Kesteren et al. (2015) developed a kinetic model based on blood and tissue concentrations in time of two different engineered SAS types (NM-200 and NM-203; Appendix B) that were orally and intravenously administered, for rats. They extrapolated the model to humans using allometric scaling to all the constants including distribution constants, to estimate the silicon concentration in liver in humans for average-to-worst-case dietary exposure at steady state. The estimated silicon concentration in human liver was at a similar level as the measured or estimated liver concentrations in animal studies in which adverse effects were reported. The Panel noted that the experimental data, which were used to estimate the model parameters, i.e. the constants were not published until October 2017. The Panel noted furthermore that the estimates for the distribution constants were characterised by a high uncertainty, and that important details of the model were not given. Thus, the uncertainty would not allow drawing firm conclusions about the concentration in the human liver.

In an unpublished report made available to EFSA (Van der Lee et al., 2016 (Documentation provided to EFSA n. 64)), the total content of silicon and silicon dioxide particles were determined in human liver and spleen obtained from organ donors who donated their bodies to science. The study was conducted with an inductively coupled plasma high-resolution mass spectrometer (ICP-HRMS) operated in a standard and a single particle inductively coupled plasma high-resolution mass spectrometry (spICP-HRMS) mode. Silicon dioxide particles were identified by scanning electron microscopy equipped with an energy dispersive X-ray detector. The ICP-HRMS method used for the determination of total silicon demonstrated a poor recovery of about 12%; therefore, the analytical results for total silicon were only indicative. The silicon dioxide particles were detected only in 4 out of the 15 liver samples and in 8 out of the 15 spleen samples, where the limit of detection of the particles was above the nanoscale (170 nm). The Panel noted that, despite the fact that the summary of the report referred to 'nanoparticles', the applied analytical method (spICP-HRMS) was not able to measure particles in the nano-range.

Summary on ADME

Overall, in the few available studies in animals, after ingestion of fumed or precipitated SAS, the silicon content of the liver and kidney was slightly increased. In humans, there was little indication of absorption of silicon after ingestion of SAS. The ECETOC (2006) report concluded that 'In contrast to crystalline silica, SAS is soluble in physiological media and soluble chemical species are formed which are eliminated via the urine without modification after intestinal resorption'. The Panel noted that this was not supported by experimental data apart from limited human studies with few individuals where less than 0.5% of the orally applied SAS was excreted via urine, and urinary silicon was always within the range of normal physiological variation.

By using specifically engineered nano-silica, and employing various routes of administration, it was shown that most of the material was excreted in the faeces but that a small proportion of material measured as silicon could be found in the liver, kidney, spleen and lung indicating limited absorption. The Panel noted that due to methodological difficulties, in particular, during processing of the samples used for the determination of the presence of nanoparticles, it is often difficult to conclude on the actual quantity of nanoparticles that can be present in various organs.

It has been reported that nanosized particles of silicon dioxide can be present in different powdered foods. By using different model fluids mimicking the various steps of the GIT, it was shown that the proportion of nanosized silicon dioxide which may be released from the food is dependent on the conditions in the GIT (pH, electrolyte concentration, etc.), on the initial content of nanoparticles in the sample added to the food, and on the form of the food. However, there is uncertainty regarding the extent to which nanosized particles can come in direct contact with the cells of the GIT *in vivo*.

3.6.2. Acute toxicity

Studies with *SAS*

The acute oral toxicity of precipitated silica (Sident 9, Appendix A) was tested in male and female Wistar rats according to OECD Guideline 401 (ASTA, 1990 (Documentation provided to EFSA n. 2); Degussa AG, 1990 (Documentation provided to EFSA n. 22)). Five rats per sex were gavaged with 5,100 mg/kg bw of the test substance suspended in vehicle (1% carboxymethyl cellulose in water; application volume: 21 mL/kg bw). No clinical signs were detected during the 14-day post exposure

observation period; the weight gain of rats was 'normal'. No findings were reported at necropsy. The LD_{50} in this study was > 5,100 mg/kg bw.

In a study using a protocol comparable to OECD Guideline 401, five male and five female SD/N BR rats per group were orally gavaged with precipitated silica (Zeosyl 113; Appendix A). The test compound was mixed with water to yield a 33% suspension. Doses of 10, 12.6, 15.8 or 20 g/kg bw were tested. No clinical symptoms were reported except white faeces at day 1 (reversible after 2 days). All animals gained weight. Necropsy after the 14-day observation period revealed no pathological effects. The LD_{50} of this test compound was > 20,000 mg/kg bw (JM Huber Corporation, 1978 (Documentation provided to EFSA n. 48)).

Silica gel (Syloid 244, Appendix A) was tested in a study on acute oral toxicity (Grace, 1976 (Documentation provided to EFSA n. 45)). Five male and five female Sprague–Dawley rats received via gavage 31,600 mg/kg bw (no further details). No clinical signs were observed during the 2-day post exposure observation period. No macroscopic changes were found at necropsy. The LD_{50} was > 31,600 mg/kg bw.

In a feeding study, 10 male and 10 female Wistar rats ingested the test substance mixed with the diet (ratio of 1:4 by weight) during a 24-h period; the dose in all experiments was 10,000 mg/kg bw. The authors tested 42 substances including different precipitated and fumed silica (e.g. AEROSIL (130, 150, 200, 300, 380, OX 50), Ultrasil VN 2 and VN 3, Sident 3, Sipernat 22, 30 or 42, Silteg AS 7 and AS 9, Appendix A). None of these substances induced any signs of intoxication during the 14-day post-exposure observation period except increased size of faecal pellets due to the indigestibility of the test substances. No gross abnormalities were found at necropsy. The LD_{50} of all tested substances was > 10,000 mg/kg bw (Spanjers and Til, 1979 (Documentation provided to EFSA n. 61)).

Suspensions of fumed silica (AEROSIL 200; Appendix A) in water (containing 1% methyl-hydoxyethyl cellulose 300 P) were given via gavage to groups of 10 male and 10 female Sprague–Dawley rats at dose levels of 2,000 or 3,300 mg/kg bw (Leuschner, 1977 (Documentation provided to EFSA n. 53)). No clinical signs were noted during the 28-day observation period following administration. No relevant effects were reported on body weight gain and food consumption. No particular findings were found at necropsy. The LD_{50} was > 3,300 g/kg bw.

The acute oral toxicity of a silica gel (Syloid 244; Appendix A) was tested in adult DKK mice and Wistar rats. The LD_{50} was > 4,000 mg/kg bw for both species (Saruta et al., 1969; study in Japanese, data from an abstract).

The acute oral toxicity of two fumed silica (CAB-O-SIL M-5 or F-2; Appendix A) was also tested in male Swiss mice (Cabot, 1964 (Documentation provided to EFSA n. 5)). Ten animals received via gavage one of the two test substances in corn oil at dose levels up to 3,160 mg/kg bw. No signs of toxicity were seen in any animal during the study and no macroscopic lesions were detected upon necropsy after 14 days of observation. The LD_{50} was > 3,160 mg/kg bw.

Studies with intentionally engineered nano-SAS

In the He et al. (2011) study, all the ICR mice, treated as previously described in Section 3.6.1, survived well 1 month after being injected intravenously with MSN and PEG–MSN samples (5 mg/kg), and no pathological abnormality was observed in both gross and microscopic histological examinations.

In the study by Fu et al. (2013) (see Section 3.6.1 for details), at 24 h and 7 days after injection with different administration routes of silica particles with an average size of 110 nm, the mice were sacrificed and various organs collected. No abnormal behaviours were observed. Silica nanoparticles did not induce any changes in the appearance and micro-morphology of the liver, spleen, kidney and lung at 24 h and 7 days by different exposure routes at 50 mg/kg. Silica nanoparticles caused no injury to duodenum, jejunum, ileum, mesenteric lymph nodes and Peyer's Patch after exposure by oral exposure. However, some inflammation was observed around the injection sites of quadriceps femoris and hypodermic tissues at 24 h. Furthermore, the inflammatory response became more serious 7 days post-injection.

Overall, the Panel noted that there was evidence that SAS and engineered nano-silica had a low acute toxicity after oral administration. Only when engineered nano-silica was injected via other routes (Nemmar et al., 2016; Chan et al., 2017) some minor effects were reported. These effects were, however, not considered for hazard identification because the route of administration was not relevant for the use of SAS as a food additive.

3.6.3. Short-term and subchronic toxicity

Studies with SAS

In the van der Zande et al. (2014) study, (see full description of the study protocol in Section 3.6.1 ADME) with Sprague–Dawley rats, biochemical and immunological markers in blood and isolated cells did not indicate toxicity, but histopathological analysis, showed an increased incidence of liver fibrosis after 84-days of exposure, which reached significance only in the treated animals with NM-202. This observation was accompanied by a moderate, but significant increase in the expression of fibrosis-related genes in the liver samples. The authors concluded that: 'the liver effects observed in the present study are much lower in severity and incidence than in previous studies in which silica nanoparticles (produced by precipitation) had been systemically administered. The observed liver effects appeared to be mild and were not accompanied by changes in biochemical markers in blood, but supported by mild changes in transcriptome analysis data from the liver'. They finally concluded that: 'Additional studies seem warranted to further evaluate the biological relevance of the observed fibrosis in liver of NM-202 exposed animals'. The authors also concluded that in these studies, dose-effect relations should be studied at lower dosages, more representative of the current exposure of consumers, since only the highest dosages (1,000 and 2,500 mg/kg bw per day of NM-202 or SAS, respectively) were used for the 84-day study. The Panel noted that the dose of SAS used in this study was 125 times higher than the estimated mean human exposure to the food additive E 551 in the non-brand-loyal scenario for the most exposed population (children). The data reported in this study have been discussed (Krueger et al., 2017), and in particular, it was considered that the definition of fibrosis as well as the statistical analysis needed reappraisal. The Panel considered that the data from this study were not sufficiently robust to conclude on the reliability and relevance of the effects reported.

The toxicity of precipitated silica (Sipernat 22, Appendix A) was studied in a feeding study, performed with a protocol close to the current OECD Guideline No 408, with groups of 10 male and 10 female Wistar rats, which received the test substance in the diet at concentration levels of 0%, 0.5%, 2% or 8 % (equal to 0, 300–330, 1,200–1,400 and 4,000–4,500 mg/kg bw per day) for a period of 13 weeks (Degussa, 1981 (Documentation provided to EFSA n. 20); Til et al., 1981 (Documentation provided to EFSA n. 62)). Blood samples of all rats were collected at week 13. No adverse effects were reported in any measured parameters. The enlargement of the caecum in males and females of the high-dose group was considered to be related to the intake of high amounts of the inert test item since no histopathological findings were noted. This hold also for the increased food consumption accompanied by decreased food efficiency at the high-dose level. According to the authors, the no observed adverse effect levels (NOAEL) in this study was 8% (equal to 4,000 mg/kg bw per day and 4,500 mg/kg bw per day for male and female, respectively), the highest dose tested. The Panel agreed with this NOAEL.

In a subchronic feeding study, male and female albino rats (no further details) received for 90 days a diet containing 0% (control), 1%, 3% or 5 % (equal to 700, 2,100, and 3,500 mg/kg bw per day) fumed silica (CAB-O-SIL; Appendix A) (Cabot, 1958 (Documentation provided to EFSA n. 4)). No clinical signs of toxicity were noted during the exposure period. No effects occurred on body weight gain, food consumption or survival. Necropsy of rats after 45 or 90 days of treatment revealed no test item related effects. Histopathology of 'representative' rats of control and treatment groups after 90 days of exposure did not reveal any significant findings. The silicon content in the liver, kidney, spleen, blood, and urine of high dose rats treated for a period of 45 or 90 days was not increased compared to both control groups (no further information available). According to the author, the NOAEL in this study was 3,500 mg/kg bw per day, the highest dose tested. In the absence of information about haematological and clinical chemistry parameters in the document available, the Panel considered that no reliable NOAEL could be identified from this study.

Silica gel (Syloid 244; Appendix A) was tested in a feeding study in rats (Grace GmbH, 1975a, 1975b (Documentation provided to EFSA n. 43 and 44)). Groups of 12 male and 12 female CD rats were exposed for 6 months to diet containing 0%, 3.2% or 10% Syloid 244 (equal to 0, 2,170 and 7,950 mg/kg bw per day in males and 0, 2,420 and 8,980 mg/kg bw per day in females). Blood and urine samples of four rats per sex per dose were collected for haematology and urinalysis (parameters: pH, albumin, glucose, ketones, sediment, bilirubin, occult blood) at 6, 13 and 26 weeks after initiation of the exposure period. At termination, organs were weighed and a histopathological examination was performed (data on examined organs not available from the original report). In a study summary

report (Grace GmbH, 1975b (Documentation provided to EFSA n. 44)), the authors reported no treatment-related adverse effects concerning any examined parameter. In the original report (Grace GmbH, 1975a (Documentation provided to EFSA n. 43)), the authors mentioned a deviation ≥ 20% of the weights of adrenal and pituitary glands (no further details) which was statistically not significant but no adverse effects were found at histopathology of the adrenal gland. Because of the missing information (no data available about histopathology of pituitary gland and data on body weight gain, food consumption and haematology were not available from the result section of the original report), the Panel did not identify a NOAEL from this study.

Within the framework of a feeding study on reproductive toxicity (see also Section 3.6.6), five male and five female Wistar rats received fumed silica (AEROSIL, not further specified) via the diet for 6 months; controls were fed the basal diet (Leuschner, 1963a,b (Documentation provided to EFSA n. 51 and 52)). The authors calculated a mean dose of 500 mg/kg bw per day. No clinical signs were noted and body weight gain and food consumption were comparable to control values. Monthly haematological examinations revealed no relevant effects (parameters observed were: number of erythrocytes and leucocytes, differential white blood cell count (WBC), haemoglobin (Hb)). The author reported that no gross changes were found at necropsy and organ weights did not show 'certain differences' between control and treatment groups (no statistical evaluation performed). Data on histopathology were not available.

Studies with intentionally engineered nano-SAS

Mice

In the study by Yoshida et al. (2014), mice were orally administered for 28 days with amorphous silica particles with particle sizes of 70, 300 and 1,000 nm (nSP70, mSP300, and mSP1000, respectively) (see Section 3.6.1 for description of the experimental material used). Haematological, histopathological and biochemical analyses showed no significant differences between control mice and mice treated with the silica particles.

So et al. (2008) studied the toxic effects of nano- and microsilica particles in Balb/c and C57BL/6J (black) mice (5 animals of each sex per group) fed for 10 weeks a diet containing 1% of micron (0.5–30 μm) or nanosized (30–90 nm) silica particles. The particles were prepared from rice husks; in a first step, microsized particles (0.5–30 μm) were prepared from rice husk, then nanoparticles (30–90 nm) were produced by ultrasonication and stabilisation. Serum biochemistry and haematological examination was performed. The only reported effect was an increased (doubling) serum ALT of BALB/c and C57BL/6 mice fed the diet with nanosized and microsized silica. The Panel noted that this study had a number of limitations (low number of animals, poor characterisation of the particle size, possible presence of crystalline silica owing to the origin of the material used in this study).

Rats

A 28-day study (Fraunhofer, 2011 (Documentation provided to EFSA n. 36)) compliant with good laboratory practice (GLP), was performed in Wistar (WU) rats and according to the current OECD TG 407; however, only males were used. The rats were administered by gavage a suspension of nano precipitated silica (NM-200; JRC, 2013; Appendix B) freshly prepared daily in carboxymethylcellulose (0.5% in deionised water). The animals (five per group) received 0, 100, 300 or 1,000 mg/kg bw per day for 28 days; an additional recovery group received 1,000 mg/kg bw per day and was kept for 14 days. At the end of the treatment period, silicon was determined (ICP-MS) in the blood, liver and kidneys. The presence of particles was assessed by TEM in the liver, kidneys, and mesenteric nodes (specimen were embedded in epoxide resin and ultrafine sections were observed by TEM). A range of clinical chemistry and haematology parameters were evaluated as well as locomotor activity. At the end of the study, no significant difference was reported between the treated and control groups. Silicon concentration in blood, liver and kidneys was comparable between treated and control animals. Occasionally electron dense structures were found in the cytoplasm of different cells in both treated and untreated animals. These structures did not show the shape and appearance of amorphous silica. However, the authors considered that though similar material has also been found in the control group, it could not be ruled out completely that some of these structures found in the SAS treated group might have been nanoparticles. The authors concluded that this material did not cause any substance-related effects in doses up to 1,000 mg/kg bw per day after oral exposure for 28 days in male Wistar rats. The Panel agreed with this conclusion.

Yun et al. (2015) examined the systemic toxicity of silica nanoparticles (primary particle size 12 nm (TEM) by oral administration to Sprague–Dawley rats (12/sex and per group). In a preliminary experiment, rats (five per sex and group) were administered orally with doses of 490, 980, or 1,960 mg/kg bw nano silicon dioxide for 14 days. There were no dose-related changes in mortality, clinical observations, body weight, haematology, serum biochemistry and organ weights. Then, a 13-week study was performed according to the OECD Test Guideline 408. Based on the results of the 14-day study, the animals were orally administered doses of 0, 245, 490 or 980 mg/kg bw per day. The silica nanoparticles did not induce any dose-related changes in a number of parameters (urinalysis, haematology, serum biochemistry, organ weights, histopathological examination of heart, lung, spleen, thymus, kidney adrenal gland, testis, ovary, brain and pituitary gland), associated with the systemic toxicity up to the highest dose tested of 980 mg/kg bw per day. The authors also reported that silica was not systemically distributed in tissues and that most of the ingested silica was excreted in the faeces.

Overall, the subchronic toxicity of SAS, including food-grade material, appeared low. One study reported that at high doses (2,500 mg/kg bw per day) pyrogenic SAS (E 551) induced limited signs of liver fibrosis and accumulation in the spleen of rats after 84 days of exposure; however, the Panel considered these data not robust enough. In a subchronic study comparable to OECD Guideline 408, no adverse effects were detected in rats after feeding a diet containing up to 8% precipitated SAS for a period of 13 weeks. The NOAEL in this study was 8% (equal to 4,000 mg/kg bw per day and 4,500 mg/kg bw per day for male and female, respectively), the highest dose tested. Similarly, no adverse effects were reported in rats after feeding silica gel for 6 months at dose levels up to 10% in the diet (equal to 7,950 mg/kg bw per day in males and 8,980 mg/kg bw per day in females). A further subchronic feeding study with rats receiving a diet containing up to 5% (equal to 3,500 mg/kg bw per day) fumed silica for 90 days supported the low subchronic toxicity of SAS.

In recent studies in rats with engineered nanosized silica and conducted according to protocols in accordance or close to OECD guidelines, the reported effects (increase absolute and relative weights of some organs mainly the liver and lung with no indication of histopathological changes) were limited when using doses up to 980 mg/kg per day, the highest dose tested.

3.6.4. Genotoxicity

The genotoxicity of SAS has been investigated in numerous *in vitro* and *in vivo* studies. The studies were identified in a focussed literature search and include those with commercial SAS, which according to industry are either used or not used as a food additive, and/or in cosmetics and pharmaceuticals. Studies with nano SAS that were considered as intentionally engineered nano-SAS were also assessed.

The Panel evaluated the reliability of the studies and the relevance of the results. This was based on the relevance of the genetic endpoint investigated and the reliability of the study. The relevance of the study results was classified into either high, limited or low. The study results of 'low relevance' were not further considered for the assessment of the genotoxicity.

A table on the available *in vitro* and *in vivo* genotoxicity studies with indication of their reliability and relevance is presented in Appendix I.

Studies performed with SAS used as a food additive, or in cosmetics or pharmaceuticals

In vitro *studies*

SAS used as a food additive, in cosmetics or in pharmaceuticals (see Appendix I) yielded no evidence for mutagenicity in bacterial gene mutation assays (Ames test) in studies which provided results of limited relevance (Degussa, 1983 (Documentation provided to EFSA n. 21); Cabot, 1989a (Documentation provided to EFSA n. 7); Cabot, 1994a (Documentation provided to EFSA n. 11); Cabot 1994b (Documentation provided to EFSA n. 12)). Similarly, in mammalian cells, these did not induce gene mutations at the HPRT *locus* in Chinese hamster ovary (CHO) cells in a study of high relevance (Cabot, 1990b (Documentation provided to EFSA n. 10)) and did not show clastogenic activity in two studies of limited relevance (Litton Bionetics, 1974 (Documentation provided to EFSA n. 54); Cabot, 1990a (Documentation provided to EFSA n. 9)).

In vivo *studies*

In the *in vivo* studies which all provided results of limited relevance, SAS used as a food additive, in cosmetics or in pharmaceuticals proved to be negative for the induction of chromosomal aberration

and dominant lethal effects in rats (Litton Bionetics, 1974 (Documentation provided to EFSA n. 54)), or gene mutation in the *ex vivo* gene mutation assay at the HPRT locus (Johnston et al., 2000).

The available *in vitro* and *in vivo* studies on the induction of gene mutations and chromosomal aberrations did not indicate a potential for genotoxicity. An uncertainty was, however, due to the fact that all study results on the induction of chromosomal aberrations were of 'limited relevance'.

Studies performed with SAS that are not used as a food additive nor used in either cosmetics or pharmaceuticals

In vitro *studies*

SAS not used as a food additive nor used in either cosmetics or pharmaceuticals (see Appendix I) yielded no evidence for mutagenicity in the bacterial gene mutation assays (Ames test) in one study of high relevance (Mortelmans and Griffin, 1981 (Documentation provided to EFSA n. 59)), in three studies of 'high/limited' relevance (Wacker 1988a (Documentation provided to EFSA n. 65); Wacker 1988b (Documentation provided to EFSA n. 66); Prival et al., 1991) and in one study of limited relevance (Cabot, 1995a (Documentation provided to EFSA n. 13)). In contrast, in mammalian cells, SAS not used as a food additive nor used in either cosmetics or pharmaceuticals induced micronuclei in a study of high relevance (Liu et al., 1996) and equivocal results for the same end point in a study of limited relevance (Decan et al., 2016). Positive findings were also observed for the induction of DNA fragmentation in the alkaline comet assay in different rodent and human cell lines in two studies of limited relevance (Zhong et al., 1997; Gerloff et al., 2009). Negative findings were only observed in the alkaline comet assay in a study of limited relevance (Gehrke et al., 2012).

No *in vivo* studies were available.

Based on the available *in vitro* studies, there was no concern with respect to the induction of gene mutations. However, there were some indications for structural and/or numerical chromosomal aberrations.

Studies performed with intentionally engineered nano-SAS

In vitro *studies*

For intentionally engineered nano-SAS, no mutagenicity studies in bacteria were available. In mammalian cells, negative findings were observed for the induction of gene mutation in a study of high relevance (Fraunhofer, 2012a (Documentation provided to EFSA n. 37)) and in two studies of limited relevance (Park et al., 2011; Guichard et al., 2015a). Negative findings were also observed in studies evaluated to be of limited relevance for DNA fragmentation in the comet assay (Gonzales et al., 2010; Fraunhofer 2012c (Documentation provided to EFSA n. 39); Watson et al., 2014; Guichard et al., 2015a,b), for the induction of chromosomal aberrations (Fraunhofer 2012b (Documentation provided to EFSA n. 38)) and micronuclei (Vecchio et al., 2014). In contrast, positive findings in studies of 'limited relevance' were observed for DNA fragmentation in the comet assay (Yang et al., 2009; Duan et al., 2013) and for the induction of micronuclei (Park et al., 2011).

In vivo *studies*

For intentionally engineered nano-SAS, negative findings were generally observed in studies evaluated to be of limited relevance in the comet assay, in different organs/tissues including the site of contact (duodenum and colon) in rats, following the oral route of administration (Tarantini et al., 2015a) or the intratracheal one (Guichard et al., 2015a). Negative results were also observed for the induction of micronuclei in the bone marrow of rats following the oral or the intratracheal route of administration (Guichard et al., 2015a; Tarantini et al., 2015a). Equivocal results for the induction of micronuclei were observed for fumed silica (NM-203) in the rat bone marrow, following the intravenous route of administration (Guichard et al., 2015b), or in the colon of rats for fumed silica (NM-202 and NM-203) following oral administration (Tarantini et al., 2015a).

Based on the available *in vitro* studies, there was no concern with respect to the induction of gene mutations. However, there were some indications from *in vitro* studies for structural and/or numerical chromosomal aberrations. The available *in vivo* studies were not adequate to fully rule out this concern because the negative results observed in comet assays and in micronucleus assays are were of 'limited relevance' only. Additionally, there were also equivocal results of 'limited relevance' observed in *in vivo* micronucleus assays.

Studies performed with colloidal SAS

In vitro *studies*

Colloidal SAS did not show evidence for mutagenicity in the bacterial gene mutation assays (Ames test) in a study of high relevance (Kwon et al., 2014). Negative results in mammalian cells in studies of 'limited relevance' were also observed for DNA fragmentation in the alkaline comet assay (Barnes et al., 2008; Kim et al., 2010), for the induction of chromosomal aberrations (Kwon et al., 2014) and for the induction of micronuclei (Downs et al., 2012). In contrast, positive findings of 'limited relevance' were observed in studies for DNA fragmentation in the alkaline comet and alkaline unwinding assays (Mu et al., 2012; Maser et al., 2015) and for the induction of micronuclei (Tarantini et al., 2015b).

In vivo *studies*

Colloidal SAS proved to be negative in the alkaline comet assay (in studies evaluated to be of limited relevance) in the liver and the stomach of mice, following oral administration (Kwon et al., 2014), in rat lung cells following the intratracheal administration (Maser et al., 2015) and in liver, lung and white blood cells of rats following the intravenous administration of nanoparticles (55 nm) (Downs et al., 2012). Equivocal results for the comet assay were observed in rat liver, following the intravenous administration with nanoparticles (15 nm) (Downs et al., 2012). Negative results were observed for the induction of micronuclei in the bone marrow of mice following oral gavage (but no proof of bone marrow exposure) (Kwon et al., 2014), or following intratracheal administration to rats (Maser et al., 2015).

Based on the available *in vitro* studies, there was no concern with respect to the induction of gene mutations. Inconsistent results of limited relevance were observed in *in vitro* studies for chromosomal aberrations and DNA fragmentation while negative results were observed *in vivo* for these genetic endpoints. However, some uncertainty remains because only limited relevance could be assigned to these *in vivo* results.

Conclusions on genotoxicity

For SAS used as a food additive, in cosmetics or in pharmaceuticals, the available *in vitro* and *in vivo* study results, although of 'limited relevance' did not indicate any potential for genotoxicity, and overall the Panel considered that SAS used as a food additive did not raise a concern with respect to genotoxicity.

Results obtained with SAS with other uses as well as with intentionally engineered nano-SAS were not considered relevant by the Panel for the re-evaluation of silicon dioxide (E 551).

3.6.5. Chronic toxicity and carcinogenicity

Studies with SAS

Mice

In a chronic feeding study (Takizawa et al., 1988), groups of 40 male and 38–40 female B6C3F1 mice were exposed to silica gel (Syloid 244, Appendix A). The animals (5 weeks old at initiation) received for 93 weeks a diet containing 0%, 1.25%, 2.5% or 5 % of the test item (equivalent to 0, 1,875, 3,750 and 7,500 mg/kg bw per day). Interim sacrifices of 10 mice per sex per dose were performed after 6 and 12 months of treatment. Haematological (red blood cell (RBC), Hb, WBC and haematocrit (Ht)) and clinical chemistry examinations (aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), inorganic phosphorus; protein, albumin, lactate dehydrogenase (LDH), alkaline phosphatase (ALP), bilirubin, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride, blood albumin nitrogen, uric acid; creatinine and calcium) were performed of blood/serum samples of all mice at the end of the 6- and 12-month periods. At autopsy gross and microscopic examination (only hyperplastic and neoplastic lesions were reported) was performed of lungs, bronchi, heart, kidneys, liver, spleen, brain, stomach, colon, intestines, pancreas, adrenal glands, pituitary, thyroid, salivary glands, thymus, testes, prostate, bladder, ovaries, uterus, oviducts, femoral bones, mammary glands, skin and subcutis. Up to a dose of 5% in the diet, no clinical signs were noted and there were no effects of toxicological relevance on body weight (difference compared with control < 10%) and food consumption. The survival was not influenced by the treatment. Haematology and clinical chemistry determined at 6 month intervals revealed no adverse effects. No dose-related alterations of organ weights were seen. Necropsy and histopathology

showed no evidence for pathologic and carcinogenic effects (no reporting of non-neoplastic lesions and only 20 males and 20 females per group were examined after 24 months). The NOAEL identified by the Panel from this study was 5% in the diet, equivalent to 7,500 mg/kg bw per day, the highest dose tested.

Rats

Takizawa et al. (1988) also studied the chronic toxicity in groups of 40 male and 40–41 female Fisher rats exposed for 103 weeks (interim sacrifice after 6 and 12 months) to silica gel (Syloid 244; Appendix A) and using the same experimental design as in the mice study described above. The animals received a diet containing 0%, 1.25%, 2.5% or 5% silica gel (equivalent to 0, 625, 1,250 and 2,500 mg/kg bw per day) The treatment did not result in any clinical signs or altered food consumption and survival. No effects of toxicological relevance on body weight were reported (difference to control < 10%). Haematology and clinical chemistry revealed no treatment-related effects. No dose-related alterations of organ weights were recorded except reduced absolute liver weights (no data about relative weight) in females of the mid- and high-dose group after 12 and 24 months; body weight was reduced by less than 10%. Necropsy and histopathology showed no evidence for pathological and carcinogenic effects (no reporting of non-neoplastic lesions and only 20 males and 20 females per group were examined after 24 months). In this feeding study, the NOAEL identified by the Panel for chronic oral administration was a dose level of 5 % in the diet, equivalent to 2,500 mg/kg bw per day, the highest dose tested.

Overall, no adverse effects were induced in rats and mice after chronic oral exposure up to 2,500 mg/kg bw per day in rats and up to 7,500 mg/kg bw per day in mice, the highest doses tested. The Panel considered that these studies in mice and rats, although not performed according to the current Guidelines, indicated that SAS was not carcinogenic.

3.6.6. Reproductive and developmental toxicity

Reproductive toxicity studies with SAS

In a feeding study on reproductive toxicity (Leuschner, 1963a,b (Documentation provided to EFSA n. 51 and 52), see also Section 3.6.3 for repeated dose toxicity), males and females Wistar rats received continuously for 6 months the basal diet or a diet containing fumed silica (AEROSIL, not further specified). The authors calculated a mean dose of 500 mg/kg bw per day in the treatment groups. After a 4.5-month premating exposure period, one male was mated with five females for 14 days in each group. Mating resulted in five pregnant animals in the treatment group and five pregnant rats in the control group. In the parental generation, no clinical signs were noted and body weight gain and food consumption were comparable to control values. The number of pups per litter was not different from the control group as well as pup weight and postnatal survival at day 7 and 21. Offspring did not show clinical signs or external malformations; post-natal body weight gain was normal. No reproductive toxicity was noted but the study was limited since only one low dose was tested in a small group of pregnant rats.

Reproductive toxicity study with intentionally engineered Nano-SAS

A two-generation reproduction toxicity study was performed with nano precipitated silica (NM-200, JRC (2013), Appendix B) in compliance with OECD TG 416 and GLP (TNO, 2012 (Documentation provided to EFSA n. 63); Wolterbeek et al., 2015). Prior to dosing, NM-200 was suspended in 0.5% v/v of methylhydroxypropylcellulose in ultrapure water as a vehicle. To achieve homogeneity, the suspension was continuously stirred. The test substance was administered by gavage (dose volume 10 mL) at dose levels of 10, 30 or 100 mg NM-200/mL (equal to 100, 300 and 1,000 mg/kg bw per day) for two generations. The authors stated that the mean hydrodynamic diameter of NM-200 particles in the test suspensions ranged between 1,076 and 1,664 nm for the low-dose group (10 mg/mL) and 876–1,216 nm for the mid-dose group (30 mg/mL) while the particles in the dispersions at the highest concentration (100 mg/mL) agglomerated, resulting in a partial sedimentation. No reproductive toxicity or influence on growth and development of the offspring were observed. According to the author, the NOAEL of this study was 1,000 mg/kg bw per day, the highest dose tested. The Panel agreed with this NOAEL.

Developmental studies with SAS

Gavage studies on prenatal developmental toxicity of silica gel (Syloid 244; Appendix A) were performed in mice (FDRL, 1973a (Documentation provided to EFSA n. 30)), rats (FDRL, 1973c (Documentation provided to EFSA n. 32)), hamsters (FDRL, 1973d (Documentation provided to EFSA n. 33)) and rabbits (FDRL, 1973e (Documentation provided to EFSA n. 34)) using a similar experimental design.

Groups of 21–24 pregnant CD-1 mice were gavaged at gestation days (GD) 6–15 with 0 (vehicle, presumably water) 13, 62, 290 or 1,340 mg/kg bw per day (FDRL, 1973a (Documentation provided to EFSA n. 30)). Clinical signs in dams were recorded daily and maternal body weighed data at GD 0, 6, 11, 15 and 17. Caesarean section and necropsy of dams was performed at GD 17. The following parameters were investigated: number of abortions, live litters, implantation sites, resorptions, dead and live fetuses as well as fetal weight. All fetuses were examined for external abnormalities, and 1/3 of each litter for soft tissue malformation and 2/3 for skeletal defects. Maternal weight at GD 15 and 17 was reduced at a dose level of 1340 mg/kg bw per day (decrease > 10% of control, no statistical evaluation). However, the relevance of this finding was questionable since the initial weight of dams in this group at GD 0 was 8 % lower than in controls. The fetuses of the highest dose group showed a lower weight (control 0.90 g vs highest dose group 0.80 g) and skeletal retardation (no statistical evaluation). No other developmental abnormalities were observed. The Panel considered that in the absence of statistical evaluation the biological relevance of the reported changes cannot be evaluated.

Groups of 20–25 pregnant Wistar rats were gavaged at GD 6–15 with 0 (vehicle, presumably water) 14, 63, 290 or 1,350 mg/kg bw per day (FDRL, 1973b (Documentation provided to EFSA n. 32)). Clinical signs were recorded daily and maternal body weighed data at GD 0, 6, 11, 15 and 20. Caesarean section and necropsy of dams was performed at GD 20. The same parameters than in the study on mice were investigated. No maternal toxicity was reported as well as no developmental toxicity.

Groups of 20–22 pregnant Syrian golden hamsters were gavaged at GD 6–10 with 0 (vehicle, presumably water) 16, 74, 345 or 1,600 mg/kg bw per day (FDRL, 1973c (Documentation provided to EFSA n. 33)). Clinical signs were recorded daily and maternal body weighed data at GD 0, 8, 10 and 14. Caesarean section and necropsy of dams was performed at GD 14. The same parameters than in the study on mice were investigated. No maternal toxicity was reported as well as no developmental toxicity.

Groups of 10–15 pregnant Dutch-belted rabbits were gavaged at GD 6–18 with 0 (vehicle, presumably water) 16, 74, 345 or 1,600 mg/kg bw per day (FDRL, 1973d (Documentation provided to EFSA n. 34)). The number of live litters was 10, 11, 8, 8 and 11 for the 0, 16, 74, 345 or 1,600 mg/kg bw per day groups, respectively. One litter of the 345 mg/kg bw per day was lost during processing. Clinical signs were recorded daily and maternal body weighed data at GD 0, 6, 12, 18 and 29. Caesarean section and necropsy of dams were performed at GD 29. The authors reported data on number of abortions, live litters, corpora lutea, implantation sites, early and late resorptions, dead and live fetuses as well as fetal weight and sex ratio. All pups were examined for external abnormalities. Live fetuses were then placed for 24 h in an incubator for evaluation of post-natal survival. Thereafter, all pups were sacrificed and examined for visceral and skeletal malformations. Data on survival of dams, abortions and body weight gain during pregnancy were comparable in all groups. The number of dead fetuses was increased in some treatment groups but without any dose-relationship. In the high-dose group, the average fetal weight was reduced (31.5 g vs 37.5 g in control; no statistical evaluation). No other developmental abnormalities were observed. The Panel considered that in this study the documentation of data and the number of litters for fetopathological examination were not sufficient to reach a final conclusion.

Developmental toxicity study with intentionally engineered nano-SAS

A prenatal developmental toxicity study with precipitated nano-precipitated silica (NM-200, (JRC, 2013), Appendix B) (BASF, 2013 (Documentation provided to EFSA n. 3); Hofmann et al., 2015) was performed in Wistar rats in compliance with OECD TG 414 and GLP. Prior to dosing, the test substance was suspended in highly deionised water containing 10% fetal bovine serum. As measured using SEM, agglomerates in the suspensions were from below 100 nm and up to 3 μm. The test substance was administered by gavage at dose levels of 0, 100, 300 or 1,000 mg/kg bw per day from GD 6-19. No maternal or developmental toxicity was observed. According to the authors, the NOAEL in this study was 1,000 mg/kg bw per day, the highest dose tested. The Panel agreed with this NOAEL.

Overall, as regards SAS, no reproductive toxicity was noted in a study, which was limited since only one low dose of AEROSIL was tested in a small group of pregnant rats. Prenatal developmental toxicity studies with silica gel (Syloid 244) showed no developmental effects up to the highest doses tested (1,350 mg/kg bw per day in rats and 1,600 mg/kg bw per day in hamsters) (FDRL, 1973c,d (Documentation provided to EFSA n. 32, 33) (Documentation provided to EFSA n. 32)). However, these developmental studies were not well documented; the statistical analysis was not described and they were not performed in accordance with the current guidelines.

As regards studies with engineered nano-SAS, in a two-generation reproductive toxicity study performed by gavage in Wistar rats in compliance with OECD TG 414 and GLP (Wolterbeek et al., 2015) with nano-precipitated silica (NM-200), no reproductive toxicity or influence on growth and development of the offspring were observed. The NOAEL of this study was 1,000 mg/kg bw per day, the highest dose tested. A prenatal developmental toxicity study in rats in compliance with OECD TG 414 and GLP (Hofmann et al., 2015) with nano precipitated silica (NM-200), showed no maternal or developmental toxicity up to 1,000 mg/kg bw per day, the highest dose tested.

3.6.7. Immunotoxicity, intolerance, allergenicity

In vitro

Winter et al. (2011) compared the *in vitro* effects of amorphous fumed silica nanoparticles (from Sigma Aldrich; BET 200 m^2/g mean primary diameter of 20-80 nm; suspended in culture medium and sonicated for 30 min), with fine crystalline silica on dendritic cells. Amorphous silica nanoparticles, as well as crystalline silica led to an up regulation of MHC-II, CD80 and CD86 on dendritic cells. Furthermore, these particles activated the inflammasome, leading to significant interleukin (IL)-1β-secretion by (dendritic) cells isolated from wild-type (WT) but not from caspase-1- or from NLRP3-deficient mice. Both amorphous silica nanoparticles and crystalline silica induced apoptosis.

Kusaka et al. (2014) studied the relationship between the particle size of silica (from Micromod Partikeltechnologie GmbH*;* 30, 1,000, 3,000, and 10,000 nm) and phagocytosis, inflammasome activation, IL-1β secretion, cell death in mouse bone marrow-derived macrophages cells *in vitro*. Irrespective of diameter size, silica particles were efficiently internalised via an actin cytoskeleton-dependent pathway and induced caspase-1, but not caspase-11, activation. Silica particles with a diameter 30–1,000 nm induced lysosomal destabilisation, cell death and IL-1β secretion at markedly higher levels than did 3,000-10,000 nm silica particles.

Di Cristo et al. (2016) studied the effects two preparations of fumed silica nanoparticles (NM-203; JRC, 2013, Appendix B) and precipitated silica nanoparticles (NM-200; JRC, 2013, Appendix B), of comparable size, specific surface area, surface charge, and hydrodynamic radius in complete growth medium on two murine macrophage cell lines (MH-S and RAW264.7 cells). They reported 'when incubated in protein-rich fluids, NM-203 adsorbed on their surface more proteins than NM-200 and, once incubated with macrophages, elicited a greater oxidative stress, assessed from Hmox1 induction and ROS production. Fumed silica nanoparticles (NM 203) interacted with macrophages more strongly than the precipitated NM-200 and triggered a more evident inflammatory response, as assessed by nitric oxide synthase 2 induction, NO production and the secretion of tumour necrosis factor (TNF)-α, IL-6 and IL-1β'. The authors concluded that, when compared to precipitated silica nanoparticles, fumed silica nanoparticles exhibit enhanced interaction with serum proteins and cell membranes, and cause greater oxidative stress and stronger pro-inflammatory effects in macrophages. The Panel noted that the fumed and precipitated nanoparticles used in this study had different biological reactivity.

Winkler et al. (2017) studied the interaction of immature and unprimed dendritic cells (DCs from mouse bone marrow) with fumed silica (AEROSIL 380F and AEROSIL 200F; Appendix A). Once ultrasonicated then suspended in culture medium the two SAS materials formed aggregates with mean diameters of 147 and 127 nm. Internalisation of the particles by DCs did not elicit cytotoxicity, the release of IL-1α or of TNF-α. However, SAS particles activated immature DCs, and the endocytic uptake of SAS particles into these steady-state DCs lead to the induction of the pro-IL-1β precursor. According to the authors, these results demonstrated that food-grade SAS particles were able to initiate the endosomal MyD88-dependent pathogen pattern recognition and signalling pathway in steady-state DCs. The same authors had previously published a study (Winkler et al., 2016) about how food-borne particles like SAS could alter the function of dendritic cells that act as first-line sentinels in the intestinal mucosa.

Breznan et al. (2017) examined the influence of physicochemical and biological factors on the toxicity of a set of various amorphous silica nanoparticles (10-20 nm, 5-15 nm and 12 nm, from Sigma

Aldrich). In particular, they looked at the cytokines release by different cell types: human epithelial A549, human THP-1 and mouse J774A.1 macrophage cells. Despite similar primary particle size, silica nanoparticles tested had distinct cytotoxicity profiles. The pro-inflammatory potential of the silica nanoparticles in the different cell lines was variable, emphasising the role of a specific cell type in the toxicological outcome. Silica nanoparticles (12 nm) were identified as the most potent, with particle surface acidity associated with their cytotoxic and inflammatory potency across the cell lines. According to the authors: 'Associations with other SiNPs properties including dry-state agglomerate size and transition metal components highlighted the need for refined understanding of the interrelationships between the various physico-chemical properties. However, due to the heterogeneity of the physico-chemical properties of nanoparticles and their interactions in biological matrices, it remains necessary to test all particles on a case-by-case basis and to conduct targeted validations via *in vivo* animal exposure studies'.

In vivo

In the study by Yoshida et al. (2011), female BALB/c mice were intranasally exposed to ovalbumin (OVA) plus silica particle of various sizes (nanoparticles of 30 nm or 70 nm and conventional microsized particles with diameters of 300 or 1,000 nm, and the plasma levels of OVA-specific antibodies were determined. Intranasal exposure to OVA plus smaller nano-silica particles tended to induce a higher level of OVA-specific immunoglobulin (Ig) E, IgG and IgG1 antibodies than did exposure to OVA plus larger silica particles. Splenocytes from mice exposed to OVA plus nSP30 secreted higher levels of Th2-type cytokines than mice exposed to OVA alone. Taken together, these results indicated that nano-silica particles can induce allergen-specific Th2-type allergic immune responses *in vivo*. The Panel noted that there was no control groups administered only with silica particles. The Panel also noted that the possible fixation of OVA on nanoparticles may increase the immunogenicity of OVA and therefore the increased antibody response by favouring its adsorption and captation by dendritic cells.

Toda and Yoshino (2016) evaluated the effects of amorphous silica nanoparticles with a particle diameter of 30 nm (purchased from Micromod Partikeltechnologie GmbH (no details about the methodology used for measurement of the particle size); sonicated for 5 min then vortexed prior to oral administration at 0.1, 1 or 10 mg/mouse daily for four days) on immunological unresponsiveness induced in groups of 5 BALB/c male mice with oral OVA. The production of OVA-specific antibodies, splenocyte proliferation in response to OVA, and effects on T-helper (Th)-1, Th2, and Th17 responses (cytokine and IgG/IgE subclass expression) were evaluated. At the doses of 1 and 10 mg/mouse per day, silica nanoparticles increased the levels of OVA-specific IgG in OVA-tolerised mice and induced (dose related) the proliferation of OVA-immunised splenocytes in response to OVA. nSP30 also increased the expression of OVA-specific IgG1, IgE, and IgG2a, indicating stimulation of the Th1 and Th2 responses. The expression of interferon IFN-γ, IL-4 and IL-5 (Th2) and IL-17 (Th17) was also stimulated (dose-related) by silica nanoparticles in splenocytes stimulated *ex vivo* with OVA. The induction of tolerance by OVA, the production of anti-OVA IgG antibodies, and proliferation of splenocytes in response to OVA was inhibited by silica nanoparticles in conjunction with OVA and was dose-related. The silica nanoparticles enhanced Th1 and Th2 responses that might prevent the induction of oral tolerance. According to the authors, 10 mg silica nanoparticles/mouse per day significantly blocked oral tolerance induced by consumption of OVA; this dose corresponded to 30 g silica nanoparticles/day for a 70-kg reference adult. The Panel noted that this dose was much higher than the exposure estimated in this opinion for the adult population (from 20 to 200 times higher than the mean and 95th percentile exposure, respectively).

The Panel also noted that the potential of silica nanoparticles to be used as both adjuvant and vaccine delivery vehicle is currently under investigation (Navarro-Tovar et al., 2016; Russell et al., 2016).

Overall, both *in vitro* and *in vivo*, some nanoparticles of silica appeared to have several immunomodulatory effects including an adjuvant and/or carrier effect; silica particles above the nanosize being less effective. In particular, smaller particles increased the production of type 2 cytokines by splenocytes and plasma levels of specific antibodies when administered (intra-nasally) together with ovalbumin. The Panel noted that the relevance of these studies to the risk assessment of silicon dioxide as a food additive was low. This is because most of the available *in vivo* studies have been performed with intra tracheal or intra-peritoneal administration (Morishige et al., 2012; Kusaka et al., 2014), which are routes of administration not relevant for the risk assessment of the food additive, in addition the size of the particles was not representative of the food additive, and finally, the administered doses were usually very high and well above the possible exposure of human resulting from consumption of silicon dioxide used as a food additive.

3.6.8. Other studies

Effects on humans

The safety of silica gel (Syloid HC; Appendix A) was studied in six human adults (three men and three women; aged 20–51 years) with primary type II hyperlipoproteinaemia. The subjects ingested for 3 weeks the test item with the morning and evening meal, starting with a dose of 1,000 mg/day. This dose was daily increased by 1,000 mg/day, up to a final dose of 16,000 mg/day. No marked adverse side effects were observed and the substance did not markedly enhance bile acid excretion (no further information available) (Grace, 1982, as referred to by ECETOC, 2006).

***In vitro* studies on cytotoxic effects of SAS nanoparticles and their mechanisms**

The Panel reviewed a series of studies investigating the cytotoxic potential of a variety of SAS nanoparticles, which are briefly reported below.

Silica-induced apoptosis was studied in human alveolar macrophages treated with SAS (80 μg/mL; particle size 1–5 μm) *in vitro* for 6 and 24 h (Iyer et al., 1996). In contrast to parallel experiments with crystalline silica, amorphous silica did not induce apoptosis.

Napierska et al. (2009) studied the effect of monodisperse SAS particles of different sizes (16, 19, 60, 104 or 335 nm) on the viability of endothelial cells (EAHY926 cell line). The results indicated that exposure to SAS nanoparticles caused dose dependent cell damage (lactate dehydrogenase release) and decreased survival. Concentrations leading to 50% reduction cell viability increased with particle size.

Morishige et al. (2010) compared the cytotoxicity of SAS of various particle sizes (30, 50, 70, 300 or 1,000 nm) against differentiated THP-1 human macrophage-like cells and concluded that 30–70 nm particles did not induce cell death but 300 and 1,000 nm particles showed cytotoxicity. According to the authors, this was due to generation of reactive oxygen species.

Rabolli et al. (2010) studied the influence of size, surface area and microporosity of SAS nanoparticles on the *in vitro* cytotoxic activity in different cell types. The authors concluded that it was possible to predict the *in vitro* cytotoxic potential of particles on the basis of their physical–chemical characteristics determinants; however, this potential varied with the cell type, reflecting the pleiotropic interactions of nanoparticles with biological systems.

In primary human umbilical vein endothelial cells, Corbalan et al. (2011) reported that SAS nanoparticles (primary size 10, 50, 150 or 500 nm) triggered a nitric oxide/peroxynitrite imbalance inducing inflammatory and cytotoxic effects at concentrations of $\geq$ 10 μg/mL. These effects were concentration-dependent and nanoparticle size-dependent (10 nm particles being more effective).

The effect of particle size was tested in human lung submucosal cells (Calu-3 cells) exposed to SAS of various particle sizes (10, 150 or 500 nm) (Mc Carthy et al., 2012). The exposure of cells to 10 nm particles increased cytotoxicity and cell death at concentrations $\geq$ 10 μg/mL in a time- and concentration-dependent manner. No such effects were detected with particle sizes of 150 and 500 nm even at 10-fold higher concentrations. Cell death and inflammatory reactions induced by 10 nm particles were attenuated by fisetin and catalase suggesting induction of oxidative stress as a mechanism for SAS cytotoxicity.

Yoshida et al. (2012) compared three different sizes of SAS particles (70, 300 and 1,000 nm) on their cytotoxic activity in XS52 cells (a Langerhans cell-like line). Using the same experimental conditions, the small particles induced a dose-dependent significant increase in intracellular reactive oxygen species in contrast to particles with a size of 300 or 1,000 nm.

Gehrke et al. (2012) studied the cytotoxicity of SAS particles (particle size 10, 40 or 200 nm) on human colon carcinoma cells (HT29). The cytotoxic effect was found to depend on the concentration and the size of particles. Smaller particles were more cytotoxic than larger. The authors also reported that, the addition of fetal calf serum inhibited the cytotoxic effects due to agglomeration of nanomaterial.

In the Napierska et al. (2012) study, human endothelial cells (EA.hy926 cell line) were incubated with monodispersed SAS nanoparticles of two sizes (16 and 60 nm; synthesised according to the Stöber method). Cytotoxic effects were induced at doses of 50 μg/mL. Oxidative stress markers gave positive results at cytotoxic concentrations. Transmission electron microscopy revealed intracellular uptake of nanomaterial, the particles were encapsulated in endocytic vacuoles but free particles were also evident in the cytoplasm. The authors discussed that these intracytoplasmic particles were the cause for cytotoxicity via reactive oxygen species production and protein interaction.

The inflammatory effects of SAS nanoparticles (particle size 30 nm) were investigated in human peripheral blood mononuclear cells or purified human monocytes (Yang and Choi, 2013). In both cell types, a 50% reduction in cell viability accompanied by increased production of cytokines was found at a concentration of 41 μg/mL. In further experiments, the test item induced generation of mitochondrial reactive oxygen species and inflammasome formation.

In a study with human Caco-2 cells (Sakai-Kato et al., 2014), inhibition of cell viability was induced by SAS nanoparticles with a size of 50 nm at a concentration of 1 mg/mL. In contrast, no cytotoxicity was observed for particles with a size of 100 or 200 nm even at a concentration of 10 mg/mL. Cytotoxic effects of small size particles were inhibited after incubation in medium simulating fed-state intestinal fluids.

Yang et al. (2016) examined the presence of nanoparticles in silicon dioxide (E 551) and in food products, and their impacts on human gastrointestinal tract *in vitro*. XRD, XPS and TEM analysis revealed that six different samples of silicon dioxide (E 551) obtained from commercial vendors exhibited consistent morphologies as agglomerates, with size ranging from below 100 nm to 500 nm. Primary particle size was 9–26 nm within agglomerates of 0.5 to 2 μm. Their potential for amorphous silicon dioxide to dissolve in various water matrices could reach 6.8%. These samples were considered as 'pristine' silicon dioxide because they had not yet been mixed or reacted with food matrices. Ten out of 14 foods (purchased in the USA) contained silicon dioxide (E 551) with the same morphology and size as the pristine 'food-grade' silicon dioxide, at levels of 2–200 mg silicon per serving size. According to the authors, using a realistic exposure range, pristine silicon dioxide (E 551) exhibited dose–response association onto microvilli on a cell model of the human GIT, and induced production of reactive oxygen species (ROS). In another *in vitro* assay with intestinal brush border microvilli assays, a consistent inhibitory pattern where silicon dioxide (E 551) dioxide associated with microvilli and caused microvilli disruption was observed. If loss of microvilli could impact nutrient uptake in the gastrointestinal tract, the authors noted that because the entire mucosal layer is turned over every 4–5 days in mammals, the impact of silicon dioxide (E 551) on human microvilli would be minimal. The authors concluded that effects of continuous uptake of foods containing food-grade silicon dioxide needs to be further explored.

Overall, the Panel considered that these *in vitro* data, although potentially informative on some mechanisms of cytotoxicity of SAS nanoparticles, were of limited relevance for the risk assessment of silicon dioxide (E 551) used as a food additive. This was due to the use of cell types not being relevant to the GIT, the use of high doses, and poor characterisation of the particles. The Panel noted that in these studies, the cytotoxic effect was usually reported as inversely related to the particle size and dependent on the experimental conditions (dose, time of contact, preparation of the particle suspension, culture medium).

Animal studies

In the Lu et al. (2011) study, male BALB/c mice (n = 11 per group) were injected intravenously with doses of 10, 40 and 200 mg/kg bw of silica particles with diameters of 30, 70 and 300 nm (SP30, SP70, and SP300); a control group received the vehicle (deionised water at 0.1 mL/10 g). The biochemical compositions of liver tissues and serum were analysed by integrated metabonomics analysis based on gas chromatography–mass spectrometry (GC-MS) and in combination with pattern recognition approaches. Histopathological examinations and serum biochemical analysis were also performed. For the three different particles of silica, the observed effects were mainly hepatocellular necrosis and increased serum aminotransferase and inflammatory cytokines. The toxic effects were dose-dependent. No major differences were found in the response of biological systems caused by the different silica particles among the metabolite profiles. According to the authors, not only nanosized but also sub-microsized SP can cause liver injury, which was dependent on the exposure dose.

3.7. Discussion

In this opinion, the Panel did not consider data obtained with crystalline silica (an IARC class 1 carcinogen by inhalation (IARC, 1997)) because only the amorphous form of silicon dioxide is authorised as a food additive.

According to the EU specifications for silicon dioxide (E 551), the forms of SAS used as a food additive E 551 includes fumed (pyrogenic) silica and hydrated silica (precipitated silica, silica gel and hydrous silica) depending on the process (thermal or wet) used for their manufacture. The Panel noted that among the three types of silicon dioxide, SAS is the only one to be authorised as a food additive (E 551). However, among the different forms of SAS, colloidal silica is not authorised as such.

The food additive, silicon dioxide (E 551), is a material comprised of aggregated nanosized primary particles. The Panel noted that, according to information provided by industry, the size of primary particles for precipitate SAS used as a food additive E 551 range from 5 to 15 nm (measured by TEM) (Appendix A). These aggregates can further agglomerate to form larger structures. The sizes of the aggregates and agglomerates are normally greater than 100 nm. However, it cannot be totally excluded that some aggregates of primary particles could be smaller than 100 nm in size.

The Panel noted that several analytical methods are available to measure the particle size of nanomaterials (DLS, LD, TEM, SEM). These methods measure different particle characteristics, which are reflected in the different numerical size-values obtained (see Table 2).

The Panel noted that in some biological and toxicity studies (especially those conducted in the 1960–1970s) while the authors reported analysis of 'silica' content, analytical methods available at the time were only capable of measuring silicon. The Panel considered that while this was expressed as silica by the authors, it was not possible to determine whether it was silica or silicon that was measured. The Panel noted that the analysis of silicon cannot distinguish between silicon from the food additive E 551, natural presence of silicon, or silicon from other sources of silicon dioxide.

In addition a number of studies were available with chemically modified SAS particles such as some of those used by the pharmaceutical industry. These studies were not included in the present assessment as this material was clearly different from silicon dioxide (E 551) used as a food additive.

SAS

In the few studies available in animals, after oral administration of fumed or precipitated SAS, the silicon content of the liver and kidney, and occasionally in the spleen was slightly increased. Studies in rats indicated no accumulation of silicon in animals after repeated oral applications of SAS (Degussa, 1968 as referred to by ECETOC, 2006; Klosterkötter, 1969 as referred to by ECETOC, 2006).

In humans, there was little indication of absorption of SAS after ingestion; however, silicon dioxide (of unknown origin) was occasionally found in human liver and spleen tissues. Some studies reported that less than 0.5% of silicon orally applied as silicon dioxide (1,250 mg) was excreted via urine but urinary silicon was always within the range of normal physiological variation (Degussa AG, 1966 Documentation provided to EFSA n. 18); Lang, 1966 Documentation provided to EFSA n. 50); Langendorf and Lang, 1967).

Data were available on acute oral toxicity of precipitated SAS (JM Huber Corporation, 1978; Spanjers and Til, 1979; ASTA, 1990; Degussa AG, 1990 (Documentation provided to EFSA n. 48, 61, 2, 22)) and fumed (Cabot, 1964; Leuschner, 1977; Spanjers and Til, 1979 (Documentation provided to EFSA n. 5, 53, 61)), as well as silica gel (Saruta et al., 1969; Grace, 1976 (Documentation provided to EFSA n. 45)). No adverse clinical effects were induced at doses up to 20,000 mg/kg bw. Overall, there was evidence for a low acute oral toxicity of SAS.

In a valid subchronic toxicity study comparable to OECD Guideline 408, no adverse effects were detected in rats fed a diet containing up to 8% precipitated SAS (equal to 4,000 mg/kg bw per day) for a period of 13 weeks. No clinical signs and no effects on food consumption, body weight gain or food efficiency were noted. Haematology, clinical chemistry and urinalysis did not reveal any treatment related findings as well as macroscopical and microscopical pathology. At the highest dose tested (4,000 mg/kg bw per day), no adverse effects were reported (Degussa AG, 1981; Til et al., 1981 (Documentation provided to EFSA n. 20, 62)). Accordingly, no adverse effects were reported in rats fed for 6 months a diet with silica gel at doses up to 8,980 mg/kg bw per day in females (Grace, 1975a, 1975b (Documentation provided to EFSA n. 43, 44)). Another subchronic toxicity study with rats receiving a diet containing up to 3,500 mg/kg bw per day fumed silica (only limited information available) for 90 days confirmed the low toxicity of high doses (Cabot, 1958 (Documentation provided to EFSA n. 4)). Other studies used lower doses (Leuschner, 1963b (Documentation provided to EFSA n. 52)) or were not sufficient for evaluation of repeated dose toxicity (Newberne and Wilson, 1970). Overall, there was evidence for low toxicity after repeated oral administration of SAS; no adverse effects were detected even at high dose levels up to 9,000 mg/kg bw per day.

For SAS used as a food additive, the available *in vitro* and *in vivo* study results, although of limited relevance did not indicate any potential for genotoxicity and overall the Panel considered that SAS used as a food additive did not raise a concern with respect to genotoxicity.

There were some indications for induction of structural and/or numerical chromosomal aberrations *in vitro* for SAS not used as a food additive nor used in either cosmetics or pharmaceuticals. These results were not considered relevant by the Panel for the re-evaluation of silicon dioxide (E 551), since this material is not used as food additive. However, the Panel noted that their presence in the food additive cannot be excluded due to a lack of precision in the specifications for E 551.

In a long-term feeding study in rats and in mice, no adverse hyperplastic or neoplastic lesions were observed after exposure to 0%, 1.25%, 2.5% or 5% SAS (silica gel/Syloid 244) via the diet over 21 or 24 months, respectively (Takizawa et al., 1988). No effects were observed at doses equivalent to 6,500 mg/kg bw per day in mice and 2,500 mg/kg bw per day in rats, the highest doses tested. The Panel considered that these studies in mice and rats indicated that SAS was not carcinogenic; however, the precise characteristics of the test material was not fully described, in particular the description of the primary particle size.

In a feeding study in rats (Leuschner, 1963a,b (Documentation provided to EFSA n. 51, 52)), no reproductive toxicity was noted after treatment with SAS; however, the validity of these results was limited since only one dose of 500 mg/kg bw per day was tested and the group size of pregnant rats was small (n = 4–5).

Prenatal developmental toxicity studies with silica gel showed no developmental effects up to the highest doses tested (1,350 mg/kg bw per day in rats and 1,600 mg/kg bw per day in hamsters) (FDRL, 1973c,d (Documentation provided to EFSA n. 32, 33)).

Overall, the Panel noted that the SAS test items used in the biological and toxicological studies available were different in their physicochemical properties (e.g. particle size distribution). In addition, the characteristics of the test materials were not always described in sufficient detail. Given the absence of information about the particle size distribution for silicon dioxide (E 551) in the current EU specifications, the Panel considered that no SAS preparation used in any single study might be fully representative of the food additive E 551. Accordingly, the Panel considered that one major uncertainty in the risk assessment of silicon dioxide (E 551) was that different characteristics of the various SAS forms may affect their behaviour. The variety of SAS in line with the specifications and currently on the market can result in differences in surface properties and absorption of silicon dioxide (E 551). Nevertheless, despite the limitations of the toxicological database available with SAS samples closely related to the food additive E 551, there was no indication of adverse effects. However, the absence of a robust long-term study with a well-characterised food additive and following the current guidelines remained an uncertainty.

Intentionally engineered nano-SAS

Because nanoparticles of silicon dioxide are present in the food additive E 551, studies performed with specifically designed engineered nano silicon dioxide have also been included in this assessment in order to assess any toxicity associated with nanoparticles present in the food additive, provided they were prepared using amorphous silicon dioxide. The Panel noted that although engineered nano-SAS are not intended to be used as a food additive E 551, the current EU specifications for E 551 would permit their use as a food additive E 551.

Absorption of SAS nanoparticles can be highly dependent on their interaction with biomolecules such as those that are present in the food complex matrices, which may result in the formation of a corona and/or agglomeration. The Panel noted that due to methodological difficulties, in particular during processing of the samples used for the determination of the presence of nanoparticles, it was often difficult to conclude on the presence and on the actual quantity of nanoparticles that could be found in various organs. In addition, when reported by the authors, the percentage of absorbed SiO_2 is usually very low (3%). Bellmann et al. (2015) identified several significant knowledge gaps such as: physical forces, osmotic concentration, pH, digestive enzymes, and commensal microbes, inherent properties of NMs of different chemical makeup in the determination of their percent absorption through mucus and epithelial cells.

Nano-SiO_2 had a low acute toxicity after oral administration. The Panel noted some effects were reported but it was only when nano-silica was injected via routes (e.g. intravenous or intraperitoneal), which are not representative of the use of the food additive.

As regards subchronic toxicity with nano-silica, the reported effects in animals (increase absolute and relative weights of some organs mainly the liver and lung with no indication of histopathological changes) were limited, even when using doses up to 2,000 mg/kg per day.

There were some indications from *in vitro* studies for structural and/or numerical chromosomal aberrations with intentionally engineered nano-SAS. The available *in vivo* studies are not adequate to fully rule out this concern. These results were not considered relevant by the Panel for the re-evaluation of silicon dioxide (E 551), since this material is not used as a food additive. However, the Panel noted that their presence in the food additive cannot be excluded due to a lack of precision in the specifications for E 551.

In a two-generation reproductive toxicity study in Wistar rats by gavage (Wolterbeek et al., 2015) with nano-precipitated silica (NM-200), no reproductive toxicity or influence on growth and development of the offspring were observed. No adverse effects were reported up to 1,000 mg/kg bw per day, the highest dose tested.

In a recent prenatal developmental toxicity study in rats (Hofmann et al., 2015) the nano-precipitated silica (NM-200) showed no maternal or developmental toxicity up to 1,000 mg/kg bw per day, the highest dose tested.

Both *in vitro* and *in vivo*, nanoparticles of amorphous silicon dioxide have been reported to have several immunomodulatory effects including an adjuvant and/or carrier effect for allergens; particles above the nanosize range being less effective. The Panel noted that the relevance of these studies for the risk assessment of SAS as a food additive was low because most of the available studies have been performed by intratracheal or intraperitoneal administration, which are routes of administration not relevant for the use of the food additive, and the size of the particles was not representative of the food additive, and finally, the administered dose was usually very high and well above the exposure estimated in this opinion for the adult population (from 20 up to 200 times higher than the 95th percentile and the mean exposure, respectively).

Overall, the Panel noted that a number of studies have shown that, in general oral exposure to any kind of nanoparticles (Cao et al., 2016) may induce toxicological responses *in vivo*. However, in most of the toxicological studies, the consequence of the potential interaction between nanoparticles and food components in real life was ignored. All of these interactions may eventually enhance or reduce the toxicological responses induced by nanoparticles following oral exposure (Cao et al., 2016; Go et al., 2017). According to these authors, studies using nanoparticles for oral exposure may lead to misinterpretation and underestimation or overestimation of toxicity of nanoparticles, and it is necessary to assess the synergistic effects of nanoparticles in a complex system when considering the safety of nanoparticles used in food. The Panel noted that data obtained with nano silicon dioxide designed for specific purposes which tend to markedly change their surface properties (Kurtz-Chalot et al., 2017) must be interpreted cautiously as regards their relevance to evaluate the possible effects of the food additive E 551. Notwithstanding all the aforementioned considerations and evaluations, the Panel considered that to date, no adverse effects have been observed with nano-SAS in the available oral toxicity studies *in vivo*.

Dietary exposure to silicon dioxide (E 551)

Dietary exposure to silicon dioxide (E 551) from its use as a food additive was calculated according to different scenarios, as described in Section 3.4. Silicon dioxide (E 551) is authorised to be used in 22 food categories, of which FC 0, i.e. it is 'permitted in all categories of foods excluding foods for infants and young children, except where specifically provided for'. Silicon dioxide (E 551) is also authorised according the Annex III to Regulation (EC) No 1333/2008 (Parts 1, 2, 3, 4 and 5 A and B) in many other food-improving agents (additives, enzymes, flavourings) and nutrients and can as such be found in many foods via carry-over.

The Panel did not identify brand loyalty to a specific food category, and therefore, considered that the non-brand-loyal scenario covering the general population was the most appropriate and realistic scenario for risk characterisation of E 551 because it is assumed that the population is most likely to be exposed long-term to the food additive E 551 present at the mean reported use in processed food.

The Panel noted that the refined exposure estimates are based on reported uses and use levels of silicon dioxide (E 551). If current practice changes, these refined estimates may no longer be representative and should be updated.

The exposure assessments were hampered by several uncertainties (Table 7). Overall, it was considered that the exposure was overestimated due to the use levels used and assumptions made in the exposure assessment. For an elaborate discussion of the uncertainties, see Section 3.4.5.

The Panel noted that the highest exposure estimates were always much lower (at least one order of magnitude) than the NOAELs identified (the highest doses tested) in the different toxicity studies available.

4. Conclusions

Considering that:

- the EU specifications for silicon dioxide (E 551) allow for the use of SAS with various physicochemical properties,

- depending on the method used for the analytical determination of particles of silicon dioxide (including the preparation of the sample; e.g. sonication), the presence of particles in the nano-range in food and biological samples has been reported in very variable amounts,
- 'primary particles' of silicon dioxide (E 551) aggregate during the production process. The resulting aggregates (which may be in the nano-range or larger) further agglomerate in foods and/or when in contact with biological fluid,
- there is an uncertainty about the extent to which disagglomeration and/or release of primary nanoparticles of SiO_2 may occur from such agglomerates after ingestion of food containing the food additive (E 551),
- nanoparticles of SAS interact with various components of a biological milieu and are covered by a corona with a variable composition which is variable from one preparation to another,
- the highest exposure estimates (50 mg/kg bw per day) were always much lower (at least one order of magnitude) than the NOAELs identified (the highest doses tested) in the different toxicity studies available,
- due to the analytical techniques used and in the absence of clear information, it was not always possible to determine whether it was silica (SiO_2) or silicon (Si) that was measured in the biological samples,
- silicon dioxide appears to be poorly absorbed; however, silicon containing material (in some cases presumed to be silicon dioxide) was found in some tissues,
- the toxicological data available with SAS samples closely related to the food additive E 551 were used for the evaluation of the food additive,
- despite the limitations in the subchronic, reproductive and developmental toxicological studies, including studies with nano silicon dioxide, there was no indication of adverse effects,
- SAS used as a food additive does not raise a concern with respect to genotoxicity,
- there are some indications for genotoxicity for SAS not reported to be used as a food additive, in cosmetics or pharmaceuticals and for intentionally engineered nano-SAS. These results were not considered relevant by the Panel for the re-evaluation of silicon dioxide (E 551) since this material is not used as a food additive. However, their presence in the food additive cannot be excluded due to a lack of precision in the specifications for E 551,
- no carcinogenic effects were reported from chronic feeding studies at the highest doses tested of 7,500 mg silica gel/kg bw per day in mice and 2,500 mg silica gel/kg bw per day in rats,
- the material tested (silica gel, Syloid 244) in these chronic studies did not cover the full-size range of the nanoparticles that could be present in the food additive E 551 according to information provided by industry and the current EU specifications which contain no particle size limits,
- in the absence of a long-term study with nano silicon dioxide, the Panel was unable to extrapolate these results to a material complying with the current specifications for E 551, potentially containing nanoparticles,

the Panel concluded that:

- the EU specifications for silicon dioxide (E 551) are insufficient to adequately characterise silicon dioxide used as a food additive. They should include characterisation of particle size distribution using appropriate statistical descriptors (e.g. range, median, quartiles) as well as the percentage (in number and by mass) of particles in the nanoscale (with at least one dimension < 100 nm) present in silicon dioxide (E 551) used as a food additive. The measuring methodology applied should comply with the EFSA Guidance document (EFSA Scientific Committee, 2011a,b).
- from the available database there was no indication for toxicity of silicon dioxide (E 551) at the reported uses and use levels.
- due to the limitations in the available database described above the Panel was unable to confirm the current ADI 'not specified'.

The Panel considered that it would be possible to derive an ADI should the limitations in the toxicological database be reduced. The Panel noted that there were a number of approaches which could decrease these limitations which included but were not limited to a chronic toxicity study conducted according to a recognised guideline and with an adequately characterised material representative of SAS used as a food additive E 551.

5. Recommendations

The Panel recommended that:

- The European Commission considers lowering the current limits for toxic elements (arsenic, lead, mercury and cadmium) in the EU specifications for silicon dioxide (E 551) in order to ensure that the food additive will not be a significant source of exposure to these toxic elements in food.
- The European Commission considers revising the current EU specifications for E 551 to include characterisation of particle size distribution using appropriate statistical descriptors (e.g. range, median, quartiles) as well as the percentage (in number and by mass) of particles in the nanoscale (with at least one dimension < 100 nm) present in silicon dioxide (E 551) used as a food additive. The measuring methodology applied should comply with the EFSA Guidance document (EFSA Scientific Committee, 2011a,b).

Documentation provided to EFSA

1) AESGP (Association of the European Self-Medication Industry), 2016. Data on usage levels of silicon dioxide (E 551) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2016). Submitted to EFSA on 27 May 2016.
2) ASTA, 1990. Acute toxicity. Testing the acute toxicity after single oral administration in rats. Unpublished report, Sident 9. Study No. 878894. Submitted to EFSA by CEFIC, September 2011.
3) BASF, 2013. NM-200 Synthetic Amorphous Silica Prenatal Developmental Toxicity Study in Wistar Rats Oral Administration (Gavage). Project No.: 30R0032/10R024. Submitted to EFSA by CEFIC, July 2016.
4) Cabot, 1958. CAB-O-SIL. 90-day dietary feeding. Final Report dated 11th of July, 1958. Submitted to EFSA by CEFIC, September 2011.
5) Cabot, 1964. Study summary. M-5 (2L063) and F-2 – Acute oral administration - mice. Powers MB. Hazelton Laboratories, Incorporated. Fall Church, Virginia. Submitted to EFSA by CEFIC, September 2011.
6) Cabot, 1978. Toxicologic evaluation of synthetic amorphous silica particles, final report IITRI-L8034-1. Arani C, Barbera P, Levine BS and Schiff LJ, IIT Research Institute, Chicago, IL. Cabot, Boston, MA, USA. Submitted to EFSA by CEFIC, October 2017.
7) Cabot, 1989a. Robust study summary. CAB-O-SIL EH-5, Salmonella/mammalian-microsome plate incorporation mutagenicity assay (Ames test). Laboratory study T9085.501. San RHC and Springfield KA. Microbiological Associates, Rockville, Maryland, USA. Cabot, Tuscola, Illinois, USA (referred as Cabot GmbH, 1989a by ECETOC, 2006). Submitted to EFSA by CEFIC, September 2011.
8) Cabot, 1989b. Unscheduled DNA synthesis in rat primary hepatocytes. Test article CAB-O-SIL EH-5. Laboratory Study No. T9085.380. Submitted to EFSA by CEFIC, September 2011.
9) Cabot, 1990a. Robust study summary. CAB-O-SIL EH5 – Chromosome aberrations in Chinese hamster (CHO) cells. Laboratory study T9085.337. Unpublished report. Putman DL and Morris MJ. Microbiological Associates, Rockville, Maryland, USA. Cabot, Tuscola, Illinois, USA referred as Cabot GmbH, 1990c by ECETOC, 2006). Submitted to EFSA by CEFIC, September 2011.
10) Cabot, 1990b. Robust study summary. CHO/HGPRT mutation assay, CAB-O-SIL EH5. Unpublished report, Microbiol. Assoc. No. T9085.332 by Sigler CI and Harbell JW. Microbiological Associates, Rockville Maryland, USA. Cabot, Tuscola, Illinois, USA. Submitted to EFSA by CEFIC, September 2011.
11) Cabot, 1994a. *Salmonella* plate incorporation mutagenicity assay (AMES test). Test Article: CAB-O-SIL TS-530. Laboratory study G94AZ74.501. San RHC and Klug ML. Microbiological Associates, Rockville, Maryland, USA. Cabot, Tuscola, Illinois, USA (referred as Cabot GmbH, 1994d by ECETOC, 2006). Submitted to EFSA by CEFIC, October 2017.

12) Cabot, 1994b. *Salmonella* plate incorporation mutagenicity assay (AMES test). Test Article: CAB-O-SIL TS-720. Laboratory study G94BN11.501. San RHC and Pugh DL. Microbiological Associates, Rockville, Maryland, USA. Cabot, Tuscola, Illinois, USA. Report No. G94BN11.501 (referred as Cabot GmbH, 1994c by ECETOC, 2006). Submitted to EFSA by CEFIC, October 2017.
13) Cabot, 1995a. *Salmonella* plate incorporation mutagenicity assay (AMES test). Test Article: CAB-O-SIL TS-500. Laboratory study G94BN14.501. San RHC and Klug ML. Microbiological Associates, Rockville, Maryland, USA. Cabot, Tuscola, Illinois, USA (referred as Cabot GmbH, 1995j by ECETOC, 2006). Submitted to EFSA by CEFIC, October 2017.
14) Cabot, 1995b. Acute oral toxicity limit test. Product: CAB-O-SIL TS-530. Laboratory Project identification Number 3388 (referred as Cabot GmbH, 1995b by ECETOC, 2006). Submitted to EFSA by CEFIC, October 2017.
15) CEFIC, 2016a. Dossier supporting the re-evaluation of silicon dioxide (E 551) as a food additive according to Regulation (EU) No 257/2010 (Dossier prepared in accordance with the EFSA "Guidance for submission for food additive evaluations" published on 16 August 2012). Technical dossier, Volume I of VIII Date of submission: 18 July 2016, Submitted by: The Association of Synthetic Amorphous Silica Producers (ASASP), Belgium. Submitted to EFSA by CEFIC on behalf of the ASASP, July 2016.
16) CEFIC, 2016b. Re-evaluation of E551 (silicon dioxide) EFSA-Q-2011-00576- Response to EFSA's letter dated 9 June 2016- Request for additional information on particle size. Submitted to EFSA by CEFIC on behalf of the ASASP, September 2016.
17) CEFIC, 2017. Technical hearing follow-up. Re-evaluation of E551 (silicon dioxide) EFSA-Q-2011-00576- Submitted to EFSA by CEFIC on behalf of the ASASP, August 2017.
18) Degussa AG, 1966. Robust study summary. Untersuchungsbericht über den Einfluss polymerer Kieselsäuren auf die renale SiO_2-Ausscheidung. Unpublished report, Degussa AG - US-IT-No. 66-0004-DKT. Physiologisch-Chemisches Institut der Johannes-Gutenberg-Universität Mainz, Germany. Submitted to EFSA by CEFIC, September 2011.
19) Degussa AG, 1979. Study summary. Determination of the acute toxicity in rats of a number of different amorphous silicic acids, and other "white products". Unpublished report, Degussa AG - US-IT-No. 79-0004-DKT, TNO. Submitted to EFSA by CEFIC, September 2011.
20) Degussa AG, 1981. Study summary. Subchronic (13 week) oral toxicity study with SIPERNAT 22 in rats. Unpublished report, Degussa AG, US-IT-No. 81-0016-DKT. Submitted to EFSA by CEFIC, September 2011.
21) Degussa AG, 1983. Bacterial mutagenicity test on a toluene extract from AEROSIL R972. Oesch F, Pharmakologisches Institut der Universität, Mainz, Germany. Degussa, Frankfurt am Main, Germany. Report No. SP513 (referred as Degussa AG, 1983d by ECETOC, 2006). Submitted to EFSA by CEFIC, October 2017.
22) Degussa AG, 1990. Study summary. SIDENT 9 – Testing the acute toxicity after single oral administration. Unpublished report, Degussa AG - US-IT-No. 90-0038-DGT. Submitted to EFSA by CEFIC, October 2017.
23) Dr Loges Naturheilkunde neu entdecken, 2016. Data on usage levels of silicon dioxide (E 551) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2016). Submitted to EFSA on 27 April 2016.
24) EDA (European Dairy Association), 2016. Data on usage levels of silicon dioxide (E 551) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2016). Submitted to EFSA on 30 May 2016.
25) EHPM (European federation of Associations of Health Products Manufacturers), 2016. Data on usage levels of silicon dioxide (E 551) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2016). Submitted to EFSA on 31 May 2016.
26) ELC (Federation of European Food Additives, Food Enzymes and Food Cultures Industries), 2009. Food additives and nanotechnologies. Submitted to EFSA by CEFIC, September 2011.
27) Elsea JR, 1958. Study summary. CAB-O-SIL (fluffy) - Ninety-day dietary feeding. Unpublished report, July 11, from Hazleton Laboratories, Inc. Simmon, V. F., Eckford, S. L. (1979) Report F76-001. Submitted to EFSA by CEFIC, September 2011.

28) FCRA (Food Chemical Risk Analysis), 2016. Data on usage levels of silicon dioxide (E 551) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2016). Submitted to EFSA on 31 May 2016.
29) FDE (FoodDrinkEurope), 2016. Data on usage levels of silicon dioxide (E 551) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2016). Submitted to EFSA on 31 May 2016.
30) FDRL (Food and Drug Research Laboratories), 1973a. Teratology evaluation of FDA 71-48 in mice. FDA 71-48 (syloid; silica aerogel). Report PB 223808. Laboratory No. 1573. Submitted to EFSA by CEFIC, September 2011.
31) FDRL (Food and Drug Research Laboratories), 1973b. Study summary. Report PB 223808. Teratologic Evaluation of FDA 71-48 (Syloid; silica aerogel). Prep. For: FDA, U. S. Food and Drug Administration; NTIS, National Technical Information Service, U. S. Department of Commerce, USA. Submitted to EFSA by CEFIC, September 2011.
32) FDRL (Food and Drug Research Laboratories), 1973c. Teratology evaluation of FDA 71-48 in rats. FDA 71-48 (syloid; silica aerogel). Laboratory No. 1574. Submitted to EFSA by CEFIC, September 2011.
33) FDRL (Food and Drug Research Laboratories), 1973d. Teratology evaluation of FDA 71-48 in hamsters. FDA 71-48 (syloid; silica aerogel). Laboratory No. 1575. Submitted to EFSA by CEFIC, September 2011.
34) FDRL (Food and Drug Research Laboratories), 1973e. Report PB 223808. Teratology evaluation of FDA 71-48 in rabbits. FDA 71-48 (syloid; silica aerogel). Laboratory No. 1576. Submitted to EFSA by CEFIC, September 2011.
35) Fessard V, Huet S, Jarrt G, Cordelli E, Villani P, Eleuteri P, Narciso L, Tassinari R, Cubbada F, Aureli F, Andreoli C, Zijno A, Pacchierotti F and Maranghi F, 2016. *In Vivo* Genotoxicity of SiO_2 Nanoparticle after Sub-chronic Oral Exposure [Abstract, 8th International Nanotoxicology Congress]. Submitted to EFSA by CEFIC, October 2017.
36) Fraunhofer, 2011. 28-Day Oral Toxicity Study of Synthetic Amorphous Silica in Wistar (WU) Rats Fraunhofer ITEM Study No. 02G10031. Submitted to EFSA by CEFIC, July 2016.
37) Fraunhofer, 2012a. *In vitro* Mammalian Cell Mutation Test in Mouse Lymphoma L5178Y/ $TK^{+/-}$ cells with synthetic amorphous silica (NM-200). Fraunhofer ITEM Study No. 17G11007. Submitted to EFSA by CEFIC, July 2016.
38) Fraunhofer, 2012b. *In Vitro* Mammalian Chromosome Aberration Test (V79 Cells) with Synthetic Amorphous Silica (NM-200).. Fraunhofer ITEM Study No. 17G11019. Submitted to EFSA by CEFIC, July 2016.
39) Fraunhofer, 2012c. Measurement of the DNA-damaging and cytotoxic potential of synthetic amorphous silica (NM-200) in cultured primary rat alveolar macrophages. Fraunhofer ITEM Study No. 17N11540. Submitted to EFSA by CEFIC, July 2016.
40) Fraunhofer, 2014a. 14-Day Nose-Only Inhalation Toxicity Study of NM-200 (Synthetic Amorphous Silica) in Wistar WU Rats Fraunhofer ITEM Study No. 02G11001. Submitted to EFSA by CEFIC, July 2016.
41) Fraunhofer, 2014b. CEFIC-LRI N1 Project: GENOTOXICITY OF A SYNTHETIC AMORPHOUS SILICA (SAS) IN RATS (Poster). Submitted to EFSA by CEFIC, July 2016.
42) FSE (Food Supplements Europe), 2016. Data on usage levels of silicon dioxide (E 551) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2016). Submitted to EFSA on 1 June 2016.
43) Grace GmbH, 1975a. 6-Monate-Toxizitätsuntersuchung mit Syloid an Ratten bei oraler Applikation (Futteruntersuchung) - Part:1. Confidential report - unpublished, Report Inbifo A0386 1103. Submitted to EFSA by CEFIC, September 2011.
44) Grace GmbH, 1975b. Robust study summary. 6-Monate-Toxizitätsuntersuchung mit SYLOID an Ratten bei oraler Applikation (Fuetterungsversuch). Unpublished report, No. A 0386/ 1103, Part 1 - 3. Inbifo Institut für Biologische Forschung, Köln. Submitted to EFSA by CEFIC, September 2011.
45) Grace GmbH, 1976. Acute toxicity studies of Syloid 244. Huntingdon Research Center. Confidential report, HRC #N-8176-175. Submitted to EFSA by CEFIC, September 2011.

46) Grace Materials Technologies, 2016. Data on usage levels of silicon dioxide (E 551) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2016). Submitted to EFSA on 28 May 2016.
47) ICGA (International Chewing Gum Association), 2016. Data on usage levels of silicon dioxide (E 551) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2016). Submitted to EFSA on 31 May 2016.
48) JM Huber Corporation, 1978. Acute oral LD50 in the rat of ZEOSYL 113. Huntingdon Research Center. Unpublished report No.: HRC #N 783-218 (referred as JM Huber Corporation, 1978c by ECETOC, 2006). Submitted to EFSA by CEFIC, September 2011.
49) KRÜGER GmbH & Co., 2016. Data on usage levels of silicon dioxide (E 551) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2016). Submitted to EFSA on 25 May 2016.
50) Lang K, 1966. Untersuchungsbericht über den Einfluß polymerer Kieselsäuren auf die renale SiO_2-Ausscheidung. Unpublished report. Submitted to EFSA by CEFIC, September 2011.
51) Leuschner F, 1963a. Robust study summary. Über die chronische Toxizität von AEROSIL. Laboratory für Pharmakologie und Toxicologie, Hamburg, Germany. Unpublished report. Submitted to EFSA by CEFIC, September 2011.
52) Leuschner F, 1963b. Über die chronische Toxizität von AEROSIL. Bericht vom 2. January 1963. Unpublished report. Submitted to EFSA by CEFIC, September 2011.
53) Leuschner F, 1977. Robust study summary. Prüfung der akuten Toxizität von AEROSIL® 200 an Sprague-Dawley-Ratten bei peroraler Verabreichung. Unpublished report, Laboratory für Pharmakologie und Toxicologie, Hamburg, Germany 77-0004-DKT. Submitted to EFSA by CEFIC, September 2011.
54) Litton Bionetics, 1974. Mutagenic evaluation of compound FDA 71-48, silica aerogel. Unpublished report 2446 by Litton Bionetics, Kensington, Maryland, USA. Report FDABF-GRAS-311. Food and Drug Administration, Washington DC, USA [NTIS PB-245-467]. Submitted to EFSA by CEFIC, October 2017.
55) Maier M, Babick F, Retamal Marín RR and Stintz M, 2013. Does nanostructured synthetic amorphous silica disintegrate after oral uptake? SOT 2013 – 52nd Annual meeting of the Society of Toxicology, San Antonio, U.S.A., March 10-14, 2013. Submitted to EFSA by CEFIC, August 2017.
56) Maier M, Albers P, Babick F, Retamal Marín RR and Stintz M, 2014. Nanostructured synthetic amorphous silica (SAS) – absence of disintegration in intestinal environment. SOT 2014 – 53rd Annual Meeting of the Society of Toxicology, Phoenix, U.S.A. March 24-27, 2014. Submitted to EFSA by CEFIC, August 2017.
57) Maier M, Albers P, Babick F, Retamal Marín RR and Stintz M, 2015. Does nanostructured synthetic amorphous silica in industrially manufactured powered food products disintegrate after oral uptake? SOT 2015- 54th Annual Meeting of the Society of Toxicology, San Diego, U.S.A., 2015. Submitted to EFSA by CEFIC, August 2017.
58) Morfeld P, Bosch A, Weber K, Heinemann M and Krueger N, 2017. Synthetic Amorphous Silica in Food: Findings about "Liver Fibrosis" and Other Study-Related Findings in Van der Zande et al. (2014) are Questionable. EC Pharmacology and Toxicology 3.2 (2017): 49-61. Submitted to EFSA by CEFIC, October 2017.
59) Mortelmans KE and Griffin AF, 1981. Microbial mutagenesis testing of substances compound report: F76-037, Silica—silicon G-910 SCM Glidden Pigments, Lot #14-J-2 CAS #7631869 Prepared for: FDA, U. S. Food and Drug Administration; NTIS, National Technical Information Service, U.S. Department of Commerce, Springfield, VA, USA, PB89-187066. Submitted to EFSA by CEFIC, October 2017.
60) SNE (Specialised Nutrition Europe), 2016. Data on usage levels of silicon dioxide (E 551) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2016). Submitted to EFSA on 30 May 2016.

61) Spanjers MT and Til HP, 1979. Determination of the acute oral toxicity in rats of a number of different amorphous silicic acids, and other "white products". Report R 6190. TNO, Zeist. Degussa, Frankfurt, Germany. Submitted to EFSA by CEFIC, September 2011.
62) Til HP, Hollanders VMH and Beems RB, 1981. Sub-chronic (13-week) oral toxicity study with sipernat 22 in rats. Unpublished report V 81.268/201741. Civo Institutes TNO, Zeist NL. Degussa, Hanau, Germany. Submitted to EFSA by CEFIC, September 2011.
63) TNO, 2012 Oral two-generation reproduction study with NM-200 synthetic amorphous silica in Wistar rats (Vol. 1). Submitted to EFSA by CEFIC, July 2016.
64) Van der Lee M, Undas A, van Bemmel G, van Polanen Ab. 2016 The investigation of silicon and silicon dioxide in human liver and spleen tissues. Unpublished study report. Submitted to EFSA by RIKILT, May 2016.
65) Wacker Chemie GmbH, 1988a. Study to determine the ability of HDK VP KHD15 to induce mutation in four histidine-requiring strains of *Salmonella typhimurium* and tow tryptophan-requiring strains of *Escherichia coli*. Kennelly JC, Microtest Research Limited, Heslington, UK. Wacker Chemie, München, Germany. Submitted to EFSA by CEFIC, October 2017.
66) Wacker Chemie Gmb, 1988b. Study to determine the ability of HDK VP KHD50 to induce mutation in four histidine-requiring strains of *Salmonella typhimurium* and tow tryptophan-requiring strains of *Escherichia coli*. Kennelly JC, Microtest Research Limited, Heslington, UK. Wacker Chemie, München, Germany. Study No. WCG6/S. Submitted to EFSA by CEFIC, October 2017.

References

AOAC (Association of Official Agricultural Chemists), 2000. AOAC Official Method 920.08. Official Methods of Analysis of AOAC International, 17th Edition, Vol. I - Agricultural Chemicals, Contaminants, Drugs. Chapter 3.

Athinarayanan J, Alshatwi AA, Periasamy VS and Al-Warthan AA, 2015. Identification of nanoscale ingredients in commercial food products and their induction of mitochondrially mediated cytotoxic effects on human mesenchymal stem cells. Journal of Food Science, 80, N459–N464.

Barahona F, Ojea-Jimenez L, Geiss O, Gilliland S and Barrero-Moreno J, 2016. Multimethod approach for the detection and characterisation of food-grade synthetic amorphous silica nanoparticles. Journal of Chromatography A, 1432, 92–100.

Barnes CA, Elsaesser A, Arkusz J, Smok A, Palus J, Lesniak A, Salvati A, Hanrahan JP, Jong WH, Dziubaltowska E, Stepnik M, Rydzynski K, McKerr G, Lynch I, Dawson KA and Howard CV, 2008. Reproducible comet assay of amorphous silica nanoparticles detects no genotoxicity. Nano Letters, 8, 3069–3074.

Battal D, Çelik A, Güler G, Aktaş A, Yildirimcan S, Ocakoglu K and Çömelekoğlu Ü, 2015. SiO_2 Nanoparticle-induced size-dependent genotoxicity – an *in vitro* study using sister chromatid exchange, micronucleus and comet assay. Drug and Chemical Toxicology, 38, 196–204.

Bellmann S, Carlander D, Fasano A, Momcilovic D, Scimeca JA, Waldman WJ, Gombau L, Tsytsikova L, Canady R, Pereira DI and Lefebvre DE, 2015. Mammalian gastrointestinal tract parameters modulating the integrity, surface properties, and absorption of food-relevant nanomaterials. Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology, 7, 609–622.

Breznan D, Das DD, O'Brien JS, MacKinnon-Roy C, Nimesh S, Vuong NQ, Bernatchez S, DeSilva N, Hill M, Kumarathasan P and Vincent R, 2017. Differential cytotoxic and inflammatory potency of amorphous silicon dioxide nanoparticles of similar size in multiple cell lines. Nanotoxicology, 11, 223–235.

Cabot, 1994c. CAB-O-SIL TS-530, chromosome aberrations in Chinese hamster ovary (CHO) cells, laboratory study G94AZ74.330. Unpublished report. Curry PT and Schadly E. Microbiological Associates, Rockville, Maryland, USA. Cabot, Tuscola, Illinois, USA (as referred to Cabot GmbH, 1994e by ECETOC, 2006).

Cabot, 1995c. Cab-O-Sil TS-610, Salmonella plate incorporation mutagenicity assay (Ames test), laboratory study G94BN15.501. Unpublished report. San RHC and Pugh DL. Microbiological Associates, Rockville, Maryland, USA. Cabot, Tuscola, Illinois, USA (as referred to Cabot GmbH, 1995k by ECETOC, 2006).

Cabot, 1995d. CAB-O-SIL TS-500, chromosome aberrations in Chinese hamster ovary (CHO) cells, laboratory study G94BN14.330. Unpublished report. Curry PT and Schadly E. Microbiological Associates, Rockville, Maryland, USA. Cabot, Tuscola, Illinois, USA (as referred to Cabot GmbH, 1995l by ECETOC, 2006).

Cabot, 1995e. Cab-O-Sil TS-610, chromosome aberrations in Chinese hamster ovary (CHO) cells, laboratory study G94BN15.330. Unpublished report. Curry PT and Schadly E. Microbiological Associates, Rockville, Maryland, USA. Cabot, Tuscola, Illinois, USA (as referred to Cabot GmbH, 1995m by ECETOC, 2006).

Cabot, 1995f. CAB-O-SIL TS-720, chromosome aberrations in Chinese hamster ovary (CHO) cells, laboratory study G94BN11.330. Unpublished report. Curry PT and Schadly E. Microbiological Associates, Rockville, Maryland, USA. Cabot, Tuscola, Illinois, USA (as referred to Cabot GmbH, 1995f by ECETOC, 2006).

Carlisle EM, 1997. Silicon. In: O'Dell BL and Sunde RA (eds.). In Handbook of Nutritionally Essential Mineral Elements. Marcel Dekker, Inc., New York, 603–618.

Cao Y, Li J, Liu F, Li X, Jiang Q, Cheng S and Gu Y, 2016. Consideration of interaction between nanoparticles and food components for the safety assessment of nanoparticles following oral exposure: a review. Environmental Toxicology and Pharmacology, 46, 206–210.

Cavallo D, Ursini CL, Zijno A, Ciervo A, Fresegna AM, Maiello R, Palamides J, Iavicoli S and Barone F, 2016. Study of the genotoxic effects induced by two kinds of silica nanoparticles on human bronchial (BEAS-2B) epithelial cells. Journal of Clinical Toxicology, 6(Suppl.), https://doi.org/10.4172/2161-0495.C1.022

Chan WT, Liu CC, Chiang Chiau JS, Tsai ST, Liang CK, Cheng ML, Lee HC, Yeung CY and Hou SY, 2017. *In vivo* toxicologic study of larger silica nanoparticles in mice. International Journal of Nanomedicine, 12, 3421–3432.

Cho M, Cho WS, Choi M, Kim SJ, Han BS, Kim SH, Kim HO, Sheen YY and Jeong J, 2009. The impact of size on tissue distribution and elimination by single intravenous injection of silica nanoparticles. Toxicology Letters, 189, 177–183.

Contada C, Mejia J, Lozano García O, Piret J-P, Dumortier E, Toussaint O and Lucas S, 2016. Physicochemical and toxicological evaluation of silica nanoparticles suitable for food and consumer products collected by following the EC recommendation. Analytical and Bioanalytical Chemistry, 408, 271–286.

Contado C, Ravani L and Passarella M, 2013. Size characterization by Sedimentation Field Flow Fractionation of silica particles used as food additives. Analytica Chimica Acta, 788, 183–192.

Corbalan JJ, Medina C, Jacoby A, Malinski T and Radomski MW, 2011. Amorphous silica nanoparticles trigger nitric oxide/peroxynitrite imbalance in human endothelial cells: inflammatory and cytotoxic effects. International Journal of Nanomedicine, 6, 2821–2835.

Decan N, Wu D, Williams A, Bernatchez S, Johnston M, Hill M and Halappanavar S, 2016. Characterization of *in vitro* genotoxic, cytotoxic and transcriptomic responses following exposures to amorphous silica of different sizes. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 796, 8–22.

Degussa AG, 1968. Gewerbehygienisch-toxikologische Untersuchung der Kieselsäure FK700. Unpublished report. Klosterkötter W. Institut für Hygiene und Arbeitsmedizin, Essen. Degussa, Frankfurt am Main, Germany (as referred to Degussa 1968b by ECETOC, 2006.

Dekkers S, Krystek P, Peters RJ, Lankveld DP, Bokkers BG, van Hoeven-Arentzen PH, Bouwmeester H and Oomen AG, 2011. Presence and risks of nanosilica in food products. Nanotoxicology, 5, 393–405.

Demir E, Aksakal S, Turna F, Kaya B and Marcos R, 2015. *In vivo* genotoxic effects of four different nano-sizes forms of silica nanoparticles in *Drosophila melanogaster*. Journal of Hazardous Materials, 283, 260–266.

Di Cristo L, Movia D, Bianchi MG, Allegri M, Mohamed BM, Bell AP, Moore C, Pinelli S, Rasmussen K, Riego-Sintes J, Prina-Mello A, Bussolati O and Bergamaschi E, 2016. Proinflammatory effects of pyrogenic and precipitated amorphous silica nanoparticles in innate immunity cells. Toxicological Sciences: An Official Journal of the Society of Toxicology, 150, 40–53.

Docter D, Bantz C, Westmeier D, Galla HJ, Wang Q, Kirkpatrick P, Nielsen P, Maskos M and Stauber RH, 2014. The protein corona protects against size- and dose-dependent toxicity of amorphous silica nanoparticles. Bleslstein Journal of Nanotechnology, 5, 1380–1392.

Downs TR, Crosby ME, Hu T, Kumar S, Sullivan A, Sarlo K, Reeder B, Lynch M, Wagner M, Mills T and Pfuhler S, 2012. Silica nanoparticles administered at the maximum tolerated dose induce genotoxic effects through an inflammatory reaction while gold nanoparticles do not. Mutation Research, 745, 38–50.

Duan J, Yu Y, Li Y, Yu Y, Zhou X, Huang P, Sun Z, 2013. Toxic effect of silica nanoparticles on endothelial cells through DNA damage response via chk1-dependent G2/M checkpoint. PLoS ONE, 8, e62087. https://doi.org/10.1371/journal.pone.0062087

ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals), 2006. Synthetic amorphous silica. ECETOC Joint Assessment of Commodity Chemicals, JACC no. 51.

EFSA (European Food Safety Authority), 2007. Scientific opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. EFSA Journal 2007;5(1):438, 54 pp. https://doi.org/10.2903/j.efsa.2007.438

EFSA (European Food Safety Authority), 2009. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: general principles. EFSA Journal 2009;1051, 1–22. https://doi.org/10.2903/j.efsa.2009.1051

EFSA (European Food Safety Authority), 2011a. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097

EFSA (European Food Safety Authority), 2011b. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database. EFSA Journal 2011;9(3):1970, 27 pp. https://doi.org/10.2903/j.efsa.2011.1970

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources), 2009. Calcium silicate and silicon dioxide/silicic acid gel added for nutritional purposes to food supplements. EFSA Journal 2009;1132, 1132 https://doi.org/10.2903/j.efsa.2009.1132

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources), 2014. Statement on a conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010. EFSA Journal 2014;12(6):3697, 11 pp. https://doi.org/10.2903/j.efsa.2014.3697

EFSA CEF Panel, 2014. Statement on the safety assessment of the substance silicon dioxide, silanated, FCM Substance No 87 for use in food contact materials.
EFSA CONTAM Panel (EFSA Panel on Contaminants in Food Chain), 2009. Scientific Opinion on arsenic in food. The EFSA Journal 2009;7(10):1351, 199 pp. https://doi.org/10.2903/j.efsa.2009.1351
EFSA CONTAM Panel (EFSA Panel on Contaminants in Food Chain), 2010. Scientific Opinion on lead in food. The EFSA Journal 2010;8(4):1570, 151 pp. https://doi.org/10.2903/j.efsa.2010.1570
EFSA CONTAM Panel (EFSA Panel on Contaminants in Food Chain), 2012. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. The EFSA Journal 2012;10(12):2985, 241 pp. https://doi.org/10.2903/j.efsa.2012.2985
EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2014. Scientific Opinion on dietary exposure to inorganic arsenic in the European population. EFSA Journal 2014;12(3):3597, 68 pp. https://doi.org/10.2903/j.efsa.2014.3597
EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2004. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the Tolerable Upper Intake Level of Silicon.
EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2009. Scientific Opinion on the substantiation of health claims related to silicon and stimulating macrophages and increasing circulating lymphocytes (ID 358) pursuant to Article 13(1) of Regulation (EC) No 1924/2006.
EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2011. Scientific opinion on the substantiation of health claims related to silicon and protection against aluminium accumulation in the brain (ID 290), "cardiovascular health" (ID 289), forming a protective coat on the mucous membrane of the stomach (ID 345), neutralisation of gastric acid (ID 345), contribution to normal formation of collagen and connective tissue (ID 287, 288, 333, 334, 335, 1405, 1652, 1718, 1719, 1945), maintenance of normal bone (ID 287, 335, 1652, 1718, 1945), maintenance of normal joints (ID 1405, 1652, 1945), maintenance of normal appearance and elasticity of the skin (ID 288, 333), and contribution to normal formation of hair and nails (ID 334, 1652, 1719) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2011;9(6):2259. 28 pp. https://doi.org/10.2903/j.efsa.2011.2259
EFSA Scientific Committee, 2009. Guidance of the Scientific Committee on Transparency in the Scientific Aspects of Risk Assessments carried out by EFSA. Part 2: General Principles. EFSA Journal 2009;7(7):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051
EFSA Scientific Committee, 2011a. Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain. EFSA Journal 2011;9(5):2140, 36 pp. https://doi.org/10.2903/j.efsa.2011.2140
EFSA Scientific Committee, 2011b. Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379. 69 pp. https://doi.org/10.2903/j.efsa.2011.2379.
EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. https://doi.org/10.2903/j.efsa.2012.2579
EVM (Expert Group on Vitamins and Minerals), 2003. Safe upper levels for vitamins and minerals, Silicon. UK Food Standards Agency. Available online: http://www.food.gov.uk/multimedia/pdfs/vitmin2003.pdf
Fruijtier-Poelloth C, 2012. The toxicological mode of action and the safety of synthetic amorphous silica - a nanostructured material. Toxicology, 294, 61–79.
FSANZ, 2016. Potential health risks associated with nanotechnologies in existing food additives.
Fu C, Liu T, Li L, Liu H, Chen D and Tang F, 2013. The absorption, distribution, excretion and toxicity of mesoporous silica nanoparticles in mice following different exposure routes. Biomaterials, 34, 2565–2575.
Gehrke H, Fruhmesser A, Pelka J, Esselen M, Hecht LL, Blank H, Schuchmann HP, Gerthsen D, Marquardt C, Diabate S, Weiss C and Marko D, 2012. *In vitro* toxicity of amorphous silica nanoparticles in human colon carcinoma cells. Nanotoxicology, 7, 274–293.
Gerloff K, 2010. Nanoparticles and the Intestine: *in vitro* and *in vivo* investigations on genotoxic and inflammatory effects. Inaugural-Dissertation. Heinrich Heine Universität Düsseldorf, Germany. 161 pp.
Gerloff K, Albrecht C, Boots AW, Forster-Kuebler I and Schins RPF, 2009. Cytotoxicity and oxidative DNA damage by nanoparticles in human intestinal Caco-2 cells. Nanotoxicology, 3, 355–364.
Go M-R, Bae S-H, Kim H-J, Yu J and Choi S-J, 2017. Interactions between food additive silica nanoparticles and food matrices. Frontiers in Microbiology, 8, 1013. https://doi.org/10.3389/fmicb.2017.01013
Gonzalez L, Thomassen LC, Plas G, Rabolli V, Napierska D, Decordier I, Roelants M, Hoet PH, Kirschhock CE, Martens JA, Lison D and Kirsch-Volders M, 2010. Exploring the aneugenic and clastogenic potential in the nanosize range: A549 human lung carcinoma cells and amorphous monodisperse silica nanoparticles as models. Nanotoxicology, 4, 382–395.
Gonzalez L, Lukamowicz-Rajska M, Thomassen LC, Kirschhock CE, Leyns L, Lison D, Martens JA, Elhajouji A and Kirsch-Volders M, 2014. Co-assessment of cell cycle and micronucleus frequencies demonstrates the influence of serum on the *in vitro* genotoxic response to amorphous monodisperse silica nanoparticles of varying sizes. Nanotoxicology, 8, 876–884.

Grace, 1982. A study of the effect of silica hydrogel (Syloid 701) on sterol balance and on the plasma lipid and lipoprotein cholesterol levels in type IIA hyperlipoproteinemia. Unpublished report IND 18,305. Kwiterovich PO, Bachorik PS, Grundy S. 1982. John Hopkins University, School of Medicine, Baltimore, Maryland, USA. W.R. Grace, Baltimore, USA (as referred to by ECETOC, 2006).

Grunér MS, Kauscher U, Linder MB and Monopoli MP, 2016. An environmental route of exposure affects the formation of nanoparticle coronas in blood plasma. Journal of Proteomics, 137, 52–58.

Guichard Y, Maire M-A, Sebillaud S, Fontana C, Langlais C, Micillino J-C, Darne C, Roszak J, Stepnik M, Fessard V, Binet S and Gate L, 2015a. Genotoxicity of synthetic amorphous silica nanoparticles in rats following short-term exposure, part 2: intratracheal instillation and intravenous injection. Environmental and Molecular Mutagenesis, 56, 228–244.

Guichard Y, Fontana C, Chavinier E, Terzetti F, Gate L, Binet S and Darne C, 2015b. Cytotoxic and genotoxic evaluation of different synthetic amorphous silica nanomaterials in the V79 cell line. Toxicology and Industrial Health, 32, 1639–1650.

He Q, Zhang Z, Gao F, Li Y and Shi J, 2011. *In vivo* biodistribution and urinary excretion of mesoporous silica nanoparticles: effects of particle size and PEGylation. Small (Weinheim an der Bergstrasse, Germany), 7, 271–280.

Heroult J, Nischwitz V, Bartczak D and Goenaga-Infante H, 2014. The potential of asymmetric flow field-flow fractionation hyphenated to multiple detectors for the quantification and size estimation of silica nanoparticles in a food matrix. Analytical and Bioanalytical Chemistry, 406, 3919–3927.

Hofmann T, Schneider S, Wolterbeek A, van de Sandt H, Landsiedel R and van Ravenzwaay B, 2015. Prenatal toxicity of synthetic amorphous silica nanomaterial in rats. Reproductive Toxicology, 56, 141–146.

Holleman AF, 2007. Chalkogenverbindungen des Siliciums. In: Holleman AF, Wiberg E and Wiberg N (eds.). Lehrbuch Der Anorganischen Chemie. de Gruyter, Berlin. pp. 949–981.

IARC (International Agency for Research on Cancer), 1997. SILICA Crystalline silica - inhaled in the form of quartz or cristobalite from occupational sources (Group 1) Amorphous silica (Group 3).

Iler RK, 1979. The Chemistry of Silica: Solubility, Polymerization, Colloid and Surface Properties and Biochemistry of Silica. Wiley, New York.

Iyer R, Hamilton RF, Li L and Holian A, 1996. Silica-induced apoptosis mediated via scavenger receptor in human alveolar macrophages. Toxicology and Applied Pharmacology, 141, 84–92.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1974. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents. 260. Propylene glycol esters of fatty acids. WHO Food Additives Series, No. 5.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000a. Guidelines for the preparation of toxicological working papers for the Joint FAO/WHO Expert Committee on Food Additives. Geneva, Switzerland.

JECFA (Joint FAO/WHO Expert Committee on Food Additive), 2000b. Safety evaluation of certain food additives and contaminants. 976. Lead. WHO Food Additives Series, No. 44, 39p.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2015. Silicon Dioxide, amorphous (tentative). Combined Compendium of Food Additive Specifications - all Specifications Monographs 17.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2016. Compendium of food additive specifications. 82nd meeting.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2017. Eighty-fourth meeting Rome, 6–15 June 2017. Summary and conclusions.

Jin Y, Kannan S, Wu M and Zhao JX, 2007. Toxicity of luminescent silica nanoparticles to living cells. Chemical Research in Toxicology, 20, 1126–1133.

Johnston CJ, Driscoll KE, Finkelstein JN, Baggs R, O'Reilly MA, Carter J, Gelein R and Oberdörster G, 2000. Pulmonary chemokine and mutagenic responses in rats after subchronic inhalation of amorphous and crystalline silica. Toxicological Sciences, 56, 405–413.

JRC (Joint Research Center), 2013. Synthetic Amorphous Silicon Dioxide (NM-200, NM-201, NM-202, NM-203, NM-204): characterisation and Physico-Chemical Properties. JRC Repository: NM-series of Representative Manufactured Nanomaterials.

JRC (Joint Research Center), 2014. JRC Nanomaterials repository. List of representative Nanomaterials (December 2014). Available online: https://ec.europa.eu/jrc/sites/jrcsh/files/JRC%20Nanomaterials%20Repository%20-%20List%20of%20Representative%20Nanomaterials.pdf

JRC (Joint Research Center), 2016. JRC Nanomaterials repository. List of representative Nanomaterials (March 2016). Available online: https://ec.europa.eu/jrc/sites/jrcsh/files/JRC-Nanomaterials-Repository-List-of-Representative-Nanomaterials.pdf

Kim Y-J, Yu M, Park H-O and Yang SI, 2010. Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by silica nanomaterials in human neuronal cell line. Molecular and Cellar Toxicology, 6, 337–344.

Klimisch HJ, Andreae M and Tillmann U, 1997. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory Toxicology and Pharmacology, 25, 1–5. https://doi.org/10.1006/rtph.1996.1076

Klosterkötter W, 1969. Gewerbehygienisches Gutachter über die hochdisperse Kieselsäure 'HDK V 15'. Unpublished report. Institut für Hygiene und Arbeitsmedizin. Wacker Chemie, Burghausen, Germany (as referred to by ECETOC, 2006).

Knebel J, Ziemann C and Creutzenberg OH, 2014. CEFIC-LRI N1 Project: genotoxicity of a Synthetic Amorphous Silica (SAS) in Rats. Abstract #601. The Toxicologist.

Morfeld P, Bosch A, Weber K, Heinemann M, Krüeger N, 2017. Synthetic amorphous silica in food: findings about "liver fibrosis" and Other study-related findings in Van der Zande et al. (2014) are questionable. EC Pharmacology and Toxicology 3, 49–61.

Kurtz-Chalot A, Villiers C, Pourchez J, Boudard D, Martini M, Marche PN, Cottier M and Forest V, 2017. Impact of silica nanoparticle surface chemistry on protein corona formation and consequential interactions with biological cells. Materials Science and Engineering C Materials for Biological Applications, 75, 16–24.

Kusaka T, Nakayama M, Nakamura K, Ishimiya M, Furusawa E, Ogasawara K, 2014. Effect of silica particle size on macrophage inflammatory responses. PLoS ONE, 9, e92634.

Kwon JY, Kim HL, Lee JY, Ju YH, Kim JS, Kang SH, Kim YR, Lee JK, Jeong J, Kim MK, Maeng EH and Seo YR, 2014. Undetectable levels of genotoxicity of SiO_2 nanoparticles in *in vitro* and *in vivo* tests. International Journal of Nanomedicine, 9 (Suppl 2), 173–181.

Langendorf H and Lang K, 1967. Der Einfluss polymerer Kieselsaeuren auf die renale SiO2-Ausscheidung beim Menschen. Zeitschrift Ernaehrungswissenschaft, 8, 27–32.

Lee CY, 2013. Physicochemical characterisation of silica nanoparticles in complex food matrices. 12CMP056 Project Report. Available online: https://webcache.googleusercontent.com/search?q=cache:YzdAr2XEPM0J:https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/328992/Physicochemical_characterisation_of_silica_nanoparticles_in_complex_matrices.pdf+&cd=1&hl=en&ct=clnk&gl=in

Lee JA, Kim MK, Song JH, Jo MR, Yu J, Kim KM, Kim YR, Oh JM and Choi SJ, 2017. Biokinetics of food additive silica nanoparticles and their interactions with food components. Colloids Surf B Biointerfaces 150, 384–392.

Lesniak A, Fenaroli F, Monopoli MP, Aberg C, Dawson KA and Salvati A, 2012. Effects of the presence or absence of a protein corona on silica nanoparticle uptake and impact on cells. ACS Nano, 6, 5845–5857.

Lewinson J, Mayr W and Wagner H, 1994. Characterization and toxicological behavior of synthetic amorphous hydrophobic silica. Regulatory Toxicology and Pharmacology, 20, 37–57.

Lim HM, Lee J, Jeong J-H, Oh S-G and Lee S-H, 2010. Comparative study of various preparations methods of colloidal silica. Engineering, 2, 998–1005.

Liu X, Keane MJ, Zhong BZ, Ong T and Wallace WE, 1996. Micronucleus formation in V79 cells treated with respirable silica dispersed in medium and in simulated pulmonary surfactant. Mutation Research, 361, 89–94.

Lu X, Tian Y, Zhao Q, Jin T, Xiao S and Fan X, 2011. Integrated metabonomics analysis of the size-response relationship of silica nanoparticles-induced toxicity in mice. Nanotechnology 22, 055101.

Maser E, Schulz M, Sauer UG, Wiemann M, Ma-Hock L, Wohlleben W, Hartwig A and Landsiedel R, 2015. *In vitro* and *in vivo* genotoxicity investigations of differently sized amorphous SiO_2 nanomaterials. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 794, 57–74.

Mc Carthy J, Inkielewicz-Stepniak I, Corbalan JJ and Radomski MW, 2012. Mechanisms of toxicity of amorphous silica nanoparticles on human lung submucosal cells in vitro: protective effects of fisetin. Chemical Research in Toxicology, 25, 2227–2235.

Mc Clements DJ, Xiao H and Demokritou P, 2017. Physicochemical and colloidal aspects of food matrix effects on gastrointestinal fate of ingested inorganic nanoparticles. Advances in Colloid and Interface Science. 246, 165–180.

Mirshafiee V, Kim R, Park S, Mahmoudi M and Kraft ML, 2016. Impact of protein pre-coating on the protein corona compostion and nanoparticles cellular uptake. Biomaterials, 75, 295–304.

Monopoli MP, Aberg C, Salvati A and Dawson KA, 2012. Biomolecular coronas provide the biological identity of nanosized materials. (in Yoshida 2015). Nature Nanotechnology, 7, 779–786.

Morishige T, Yoshioka Y, Inakura H, Tanabe A, Yao X, Tsunoda S, Tsutsumi Y, Mukai Y, Okada N and Nakagawa S, 2010. Cytotoxicity of amorphous silica particles against macrophage-like THP-1 cells depends on particle-size and surface properties. Pharmazie, 65, 596–599.

Morishige T, Yoshioka Y, Inakura H, Tanabe A, Narimatsu S, Yao X, Monobe Y, Imazawa T, Tsunoda S, Tsutsumi Y, Mukai Y, Okada N and Nakagawa S, 2012. Suppression of nanosilica particle-induced inflammation by surface modification of the particles. Archives of Toxicology. 86, 1297–1307.

Mu Q, Hondow NS, Krzemiński L, Brown AP, Jeuken LJC and Routledge MN, 2012. Mechanism of cellular uptake of genotoxic silica nanoparticles. Particle and Fibre Toxicology, 9, 1–11.

Nabeshi H, Yoshikawa T, Matsuyama K, Nakazato Y, Matsuo K, Arimori A, Isobe M, Tochigi S, Kondoh S, Hirai T, Akase T, Yamashita T, Yamashita K, Yoshida T, Nagano K, Abe Y, Yoshioka Y, Kamada H, Imazawa T, Itoh N, Nakagawa S, Mayumi T, Tsunoda S and Tsutsumi Y, 2011. Systemic distribution, nuclear entry and cytotoxicity of amorphous nanosilica following topical application. Biomaterials, 32, 2713–2724.

NANOGENOTOX, online. Facilitating the safety evaluation of manufactured nanomaterials by characterising their potential genotoxic hazard. Final report. Project coordinator: French Agency for Food, Enviroment and Occupational Health&Safety (ANSES). Available online: https://www.anses.fr/fr/system/files/ANSES-Ft-Nanogenotox_FinalReport.pdf

Navarro-Tovar G, Palestino G and Rosales-Mendoza S, 2016 An overview on the role of silica-based materials in vaccine development. Expert Review of Vaccines, 15, 1449–1462.

Napierska D, Thomassen LC, Rabolli V, Lison D, Gonzalez L, Kirsch-Volders M, Martens JA and Hoet PH, 2009. Size-dependent cytotoxicity of monodisperse silica nanoparticles in human endothelial cells. Small (Weinheim an der Bergstrasse, Germany), 5, 846–853.

Napierska D, Thomassen LCJ, Lison D, Martens JA and Hoet PH, 2010. The nanosilica hazard: another variable entity. Particle and Fibre Toxicology, 7, 39.

Napierska D, Rabolli V, Thomassen LC, Dinsdale D, Princen C, Gonzalez L, Poels KL, Kirsch-Volders M, Lison D, Martens JA and Hoet PH, 2012. Oxidative stress induced by pure and iron-doped amorphous silica nanoparticles in subtoxic conditions. Chemical Research in Toxicology, 25, 828–837.

Nemmar A, Yuvaraju P, Beegam S, Yasin J, Kazzam EE and Ali BH, 2016. Oxidative stress, inflammation, and DNA damage in multiple organs of mice acutely exposed to amorphous silica nanoparticles. International Journal of Nanomedicine, 11, 919–928.

Newberne PM and Wilson RB, 1970. Renal damage associated with silicon compounds in dogs. Proceedings of the National Academy of Sciences, 65, 872–875.

OECD (Organisation for Economic Co-operation and Development), 2016. OECD Environment, Health and Safety Publications Series on the Safety of Manufactured Nanomaterials No. 71 SILICON DIOXIDE: SUMMARY OF THE DOSSIER.

Pacheco SE, Mashayekhi H, Jiang W, Xing B and Arcaro KF, 2007. DNA damaging effects of nanoparticles in breast cancer cells. Abstracts of the 2007 Annual Meeting of the American Association for Cancer Research.

Park MV, Verharen HW, Zwart E, Hernandez LG, van Benthem J, Elsaesser A, Barnes C, McKerr G, Howard CV, Salvati A, Lynch I, Dawson KA and de Jong WH, 2011. Genotoxicity evaluation of amorphous silica nanoparticles of different sizes using the micronucleus and the plasmid lacZ gene mutation assay. Nanotoxicology, 5, 168–181.

Peters R, Kramer E, Oomen AG, Rivera ZE, Oegema G, Tromp PC, Fokkink R, Rietveld A, Marvin HJ, Weigel S, Peijnenburg AA and Bouwmeester H, 2012. Presence of nanosized silica during in vitro digestion of foods containing silica as a food additive. ACS Nano, 6, 2441–2451.

Prival MJ, Simmon VF and Mortelmans KE, 1991. Bacterial mutagenicity testing of 49 food ingredients gives very few positive results. Mutation Research, 260, 321–329.

Rabolli V, Thomassen LC, Princen C, Napierska D, Gonzalez L, Kirsch-Volders M, Hoet PH, Huaux F, Kirschhock CE, Martens JA and Lison D, 2010. Influence of size, surface area and microporosity on the in vitro cytotoxic activity of amorphous silica nanoparticles in different cell types. Nanotoxicology, 4, 307–318.

Russell RF, McDonald JU, Lambert L and Tregoning JS, 2016. Use of the microparticle Nano-SiO2 as an adjuvant to boost vaccine immune responses in neonatal mice against influenza. Journal of Virology, 90, 4735–4744.

Sakai-Kato K, Hidaka M, Un K, Kawanishi T and Okuda H, 2014. Physicochemical properties and in vitro intestinal permeability properties and intestinal cell toxicity of silica particles, performed in simulated gastrointestinal fluids. Biochimica et Biophysica Acta, 1840, 1171–1180.

Saruta N, Ishinishi N, Kunitake E and Katori Y, 1969. Experimental study on the acute toxicity of a food additive, Syloid (amorphous silica) administered orally. Igaku Kenkyu, 39, 1–15.

Sauer F, Laughland DH and Davidson WM, 1959a. Silica metabolism in guinea pigs. Canadian Journal of Biochemistry and Physiology, 37, 183–191.

Sauer F, Laughland DH and Davidson WM, 1959b. The silica content of guinea pig tissues as determined by chemical and isotopic techniques (in ECETOC_2006). Canadian Journal of Biochemistry and Physiology, 37, 183–191. https://doi.org/10.1139/o59-020

Sayes CM, Reed KL, Glover KP, Swain KA, Ostraat ML, Donner EM and Warheit DB, 2010. Changing the dose metric for inhalation toxicity studies: short-term study in rats with engineered aerosolized amorphous silica nanoparticles. Inhalation Toxicology, 22, 348–354.

SCCS (Scientific Committee on Consumer Safety), 2015. Opinion on Silica, Hydrated Silica, and Silica Surface Modified with Alkyl Silylates (nano form). SCCS/1545/15. Revision of 29 September 2015.

SCF (Scientific Committee for Food), 1991. Opinions on the Scientific Committee for Food on: Silicate and silicon dioxide. Reports of the Scientific Committee for Food, Twenty-fifth Series.

SCF (Scientific Committee for Food), 2001. Guidance on submissions for food additive evaluations by the Scientific Committee on Food. SCF/CS/ADD/GEN/26 Final. 12 July 2001.

Sergent JA, Paget V and Chevillard S, 2012. Toxicity and genotoxicity of nano-SiO_2 on human epithelial intestinal HT-29 cell line. Annals of Occupational Hygiene, 56, 622–630.

Shah V, Taratula O, Garbuzenko OB, Patil ML, Savla R, Zhang M and Minko T, 2013. Genotoxicity of different nanocarriers: possible modifications for the delivery of nucleic acids. Current Drug Discovery Technologies, 10, 8–15.

So SJ, Jang IS and Han CS, 2008. Effect of micro/nano silica particle feeding for mice. Journal of Nanoscience and Nanotechnology, 8, 5367–5371.

Spanjers MT and Til HP, 1979. Determination of the acute oral toxicity in rats of a number of different amorphous silicic acids, and other "white products". Confidential report, Report R 6190. TNO, Zeist. Degussa, Frankfurt, Germany.

Strojan K, Leonardi A, Bregar VB, KrizÏaj I, Svete J and Pavlin M, 2017. Dispersion of nanoparticles in different media importantly determines the composition of their protein corona. PLoS ONE 12, e0169552. https://doi.org/10.1371/journal.pone.0169552

Takizawa Y, Hirasawa F, Noritomi E, Aida M, Tsunoda H and Uesugi S, 1988. Oral ingestion of syloid to mice and rats and its chronic toxicity and carcinogenicity. Acta Medica et Biologica, 36, 27–56.

Tarantini A, Huet S, Jarry G, Lanceleur R, Poul M, Tavares A, Vital N, Louro H, João Silva M and Fessard V, 2015a. Genotoxicity of synthetic amorphous silica nanoparticles in rats following short-term exposure. Part 1: Oral route. Environmental and Molecular Mutagenesis, 56, 218–227.

Tarantini A, Lanceleur R, Mourot A, Lavault M-T, Casterou G, Jarry G, Hogeveen K and Fessard V, 2015b. Toxicity, genotoxicity and proinflammatory effects of amorphous nanosilica in the human intestinal Caco-2 cell line. Toxicology In Vitro, 29, 398–407.

Tavares AM, Louro H, Antunes S, Quarré S, Simar S, De Temmerman P-J, Verleysen E, Mast J, Jensen KA, Norppa H, Nesslany F and Silva MJ, 2014. Genotoxicity evaluation of nanosized titanium dioxide, synthetic amorphous silica and multi-walled carbon nanotubes in human lymphocytes. Toxicology in Vitro, 28, 60–69.

TemaNord (Nordic Council of Ministers), 2002. E 551-3 Silicium dioxide and silicates. Food Additives in Europe 2000 - Status of safety assessments of food additives presently permitted in the EU, 535-539.

Tenzer S, Docter D, Kuharev J, Musyanovych A, Fetz V, Hecht R, Schlenk F, Fischer D, Kiouptsi K, Reinhardt C, Landfester K, Schild H, Maskos M, Knauer SK and Stauber RH, 2013. Rapid formation of plasma protein corona critically affects nanoparticle pathophysiology. (in Yoshida 2015) Nature Nanotechnology, 8, 772–781.

Toda T and Yoshino S, 2016. Amorphous nanosilica particles block induction of oral tolerance in mice. Journal of Immunotoxicology, 13, 723–728.

Uboldi C, Giudetti G, Broggi F and Gilliland D PJaRF, 2012. Amorphous silica nanoparticles do not induce cytotoxicity, cell transformation or genotoxicity in Balb/3T3 mouse fibroblasts. Mutation Research, 745, 11–20.

van der Zande M, Vandebriel RJ, Groot MJ, Kramer E, Herrera Rivera ZE, Rasmussen K, Ossenkoppele JS, Tromp P, Gremmer ER, Peters RJ, Hendriksen PJ, Marvin HJ, Hoogenboom RL, Peijnenburg AA and Bouwmeester H, 2014. Sub-chronic toxicity study in rats orally exposed to nanostructured silica. Particle and Fibre Toxicology, 11, 8.

Vance ME, Kuiken T, Vejerano EP, McGinnis SP, Hochella Jr MF, Rejeski D, Hull MS, 2015. Nanotechnology in the real world: redeveloping the nanomaterial consumer products inventory. Beilstein Journal of Nanotechnology, 6, 1769–1780.

Vecchio G, Fenech M, Pompa PP and Voelcker NH, 2014. Lab-on-a-chip-based high-throughput screening of the genotoxicity of engineered nanomaterials. Small (Weinheim an der Bergstrasse, Germany), 10, 2721–2734.

Watson C, Ge J, Cohen J, Pyrgiotakis G, Engelward BP and Demokritou P, 2014. High-throughput screening platform for engineered nanoparticle-mediated genotoxicity using CometChip technology. ACS Nano, 8, 2118–2133.

Winkler HC, Suter M and Naegeli H, 2016. Critical review of the safety assessment of nano-structured silica additives in food. Journal of Nanobiothecnology, 14, 44.

Winkler HC, Kornprobst J, Wick P, von Moos LM, Trantakis I, Schraner EM, Bathke B, Hochrein H, Suter M and Naegeli H, 2017. MyD88-dependent pro-interleukin-1β induction in dendritic cells exposed to food-grade synthetic amorphous silica. Particle and Fibre Toxicology, 14, 21.

Winter 1, Beer HD, Hornung V, Krämer U, Schins RP and Förster I, 2011. Activation of the inflammasome by amorphous silica and TiO2 nanoparticles in murine dendritic cells. Nanotoxicology, 5, 326–340.

Wolterbeek A, Oosterwijk T, Schneider S, Landsiedel R, de Groot D, van Ee R, Wouters M and van de Sandt H, 2015. Oral two-generation reproduction toxicity study with NM-200 synthetic amorphous silica in Wistar rats. Reproductive Toxicology, 56, 147–154.

Yang EJ and Choi IH, 2013. Immunostimulatory effects of silica nanoparticles in human monocytes. Immune Netw, 13, 94–101.

Yang H, Liu C, Yang D, Zhang H and Xi Z, 2009. Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by four typical nanomaterials: the role of particle size, shape and composition. Journal of Applied Toxicology, 29, 69–78.

Yang Y, Faust JJ, Schoepf J, Hristovski K, Capco DG, Herckes P and Westerhoff P, 2016. Survey of food-grade silica dioxide nanomaterial occurrence, characterization, human gut impacts and fate across its lifecycle. The Science of the Total Envirnoment, 565, 902–912.

Yoshida T, Yoshioka Y, Fujimura M, Yamashita K, Higashinsaka K, Morishita Y, Kayamuro H, Nabeshi H, Nagano K, Abe Y, Kamada H, Tsunoda S, Ithoh N, Yoshikawa T and Tsutsumi Y, 2011. Promotion of allergic immune responses by intranasally-administrated nanosilica particles in mice. Nanoscale Research Letters, 6, 195.

Yoshida T, Yoshikawa T, Nabeshi H, Matsuyama K, Hirai T, Akase T, Yoshioka Y, Itoh N and Tsutsumi Y, 2012. Amorphous nanosilica particles induce ROS generation in Langerhans cells. Pharmazie, 67, 740–741.

Yoshida T, Yoshioka Y, Takahashi H, Misato K, Mori T, Hirai T, Nagano K, Abe Y, Mukai Y, Kamada H, Tsunoda S, Nabeshi H, Yoshikawa T, Higashisaka K and Tsutsumi Y, 2014. Intestinal absorption and biological effects of orally administered amorphous silica particles. Nanoscale Research Letters, 9, 532.

Yun JW, Kim SH, You JR, Kim WH, Jang JJ, Min SK, Kim HC, Chung DH, Jeong J, Kang BC and Che JH, 2015. Comparative toxicity of silicon dioxide, silver and iron oxide nanoparticles after repeated oral administration to rats. Journal of Applied Toxicology, 35, 681–693.

van Kesteren PC, Cubadda F, Bouwmeester H, van Eijkeren JC, Dekkers S, de Jong WH and Oomen AG, 2015. Novel insights into the risk assessment of the nanomaterial synthetic amorphous silica, additive E551, in food. Nanotoxicology, 9, 442–452.

Zane A, McCracken C, Knight DA, Young T, Lutton AD, Olesik JW, Waldman WJ and Dutta PK, 2015. Uptake of bright fluorophore core-silica shell nanoparticles by biological systems. International Journal of Nanomedicine, 10, 1547–1567.

Zhang Q, Xu H, Zheng S, Su M and Wang J, 2015. Genotoxicity of mesoporous silica nanoparticles in human embryonic kidney 293 cells. Drug Testing and Analysis, 7, 787–796.

Zhong BZ, Whong WZ and Ong TM, 1997. Detection of mineral-dust-induced DNA damage in two mammalian cell lines using the alkaline single cell gel/comet assay. Mutation Research, 393, 181–187.

Zijno A, Palamides J, Andreoli C, De Berardis B and Barone F, 2016. Genotoxicity Assessment of Silica and Titania on BEAS-2B Cells in the Framework of the EU NANoREG project. Abstract. 8th International Nanotoxicology Congress. Boston, USA (as referred to by CEFIC, 2016a).

Abbreviations

AAS	atomic absorption spectrometry
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AESGP	Association of the European Self-Medication Industry
AF4	asymmetric flow-field flow fractionation
ALT/ALAT	alanine aminotransferase
ALP	alkaline phosphatase
ANS	EFSA Panel on Food Additives and Nutrient Sources added to Food
AOAC	Association of Official Agricultural Chemists
ASASP	Association of Synthetic Amorphous Silica Producers
ASAT	aspartate aminotransferase
BET	Brunauer–Emmett–Teller
BPD/R	Biocidal products directive/regulation
bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary
DC	dendritic cell
DCS	differential centrifugal sedimentation
DLS	dynamic light scattering
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EDA	European Dairy Association
EHPM	European Federation of Associations of Health Products Manufacturers
ELC	Federation of European Food Additives, Food Enzymes and Food Cultures Industries
EINECS	European Inventory of Existing Commercial Chemical Substances
ENM	engineered nanomaterial
EVM	Expert Group on Vitamins and Minerals
FAE	follicle-associated epithelium
FC	food category
FCRA	Food Chemical Risk Analysis
FCS	food categorisation system
FDA	Food and Drug Administration
FDE	Food Drink Europe
FFF-ICP-MS	field flow fractionation-inductively coupled plasma mass spectrometry
FSANZ	Food Standards Australia New Zealand
FSE	Food Supplements Europe
FSMP	foods for special medical purposes
GC	gas chromatography
GD	gestation day
GIT	gastrointestinal tract

GLP	good laboratory practice
GNPD	Global New Products Database
GRAS	Generally Recognized as Safe
HDC-ICP-MS	hydrodynamic chromatography with inductively coupled plasma mass spectrometry
HDL	high-density lipoprotein
HPLC	high-performance liquid chromatography
HPRT	hypoxanthine-guanine phosphoribosyl transferase
Ht	haematocrit
IARC	International Agency for Research on Cancer
ICGA	International Chewing Gum Association
ICP-AES	inductively coupled plasma atomic absorption spectrometry
ICP-HRMS	inductively coupled plasma high-resolution mass spectrometry
ICP–MS	inductively coupled plasma mass spectrometry
ICP-OES	inductively coupled plasma-optical emission spectrometry
Ig	immunoglobulin
IL	interleukin
IR	infrared spectrometry
ISO	International Organization for Standardization
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JRC	Joint Research Centre
LD	laser diffraction
LD_{50}	lethal dose, 50%, i.e. dose that causes death among 50 % of treated animals
LDH	lactate dehydrogenase
LDL	low-density lipoprotein
M	microfold
MA	metabolic activation
MALS	multiangle light scattering
MPL	maximum permission limit
MS	mass spectrometry
NDA	EFSA Scientific Panel on Dietetic Products, Nutrition and Allergies
NMR	nuclear magnetic resonance spectrometry
NOAEL	no observed adverse effect level
OVA	ovalbumin
OECD	Organisation for Economic Co-operation and Development
PCS	photon correlation spectroscopy
PEG–MSN	polyethylene glycol-treated mesoporous silica particle
RBC	red blood cell
ROS	reactive oxygen species
SAS	synthetic amorphous silica
SCCS	Scientific Committee on Consumer Safety
SCF	Scientific Committee on Food
SdFFF	sedimentation field flow fractionation
SEM	scanning electron microscopy
SIDS	Screening Information Dataset
SNE	Specialised Nutrition Europe
spICP-HRMS	single particle inductively coupled plasma high-resolution mass spectrometry
TEM	transmission electron microscopy
TemaNord	Nordic Council of Ministers
TNF	tumour necrosis factor
UL	upper level
UV/VIS	ultraviolet/visual (spectrometry)
WBC	white blood cell
WHO	World Health Organization
WT	wild-type
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction

Appendix A – Characteristics of synthetic amorphous silica substances used in the biological and toxicological studies (CEFIC, 2017 (Documentation provided to EFSA n. 17)); *this table is not an exclusive list of commercially available brands offered on the European market as E 551*

Name of substance	Type of substance	Nature of material	Primary particle size (nm)	Method used to determine particle size	Specific area (BET method) (m^2/g)	Commercially available as E 551	Pharma use	Cosmetics use
AEROSIL®	380	Fumed silica		TEM	380 ± 30	Yes[a]	No	Yes
	300		7		300 ± 30	No	Yes	Yes
	200		12		200 ± 25	Yes[a]	Yes	Yes
	150		14		150 ± 25	No	No	No
	130		16		130 ± 25	No	No	Yes
	Ox50		40		50 ± 15	No	No	No
	R972	Surface-treated silica				No	Yes	Yes
	COK84	It is mixture of AEROSIL® 200 and highly dispersed fumed aluminium oxide in the ratio of 5:1				No	No	No
	MOX80	It is a co-fumed oxide consisting of silicon dioxide and approximately 1% aluminium oxide, manufactured using the AEROSIL® process				No	No	No
FK	700	Precipitated silica	15,000 (aggregate)		700	No		
HDK®	V15	Fumed silica (hydrophilic grade)			150 ± 20	No		
	KHD15	Surface-treated pyrogenic silica				No		
	KHD50					No		
Sident®	9	Precipitated silica	9,000 (D_{50})	Laser diffraction	45	No	No	Yes
Sipernat®	22	Precipitated silica	115,000 (D_{50})	Laser diffraction	190	Yes	No	Yes
	50		50,000 (D_{50})		500	Yes	No	Yes
	350		4,500 (D_{50})		55	Yes	No	No
	30[b]							
	42[b]							
Zeosyl®	113	Precipitated silica				No		

Name of substance	Type of substance	Nature of material	Primary particle size (nm)	Method used to determine particle size	Specific area (BET method) (m^2/g)	Commercially available as E 551	Pharma use	Cosmetics use
Syloid®	244 FP	Silica gel	5,500	Malver	310	Yes	Yes	
	HC					No		
CAB-O-SIL®	F2[(c)]	Fumed silica				No		
	EH-5F					Yes		Yes
	M-5F/P					Yes	Yes	Yes
	TS500					No		
	TS530					No		Yes
	TS610					No		Yes
	N70TS[(d)]					No		Yes
Silcron	G-910	Silica gel				No		
Sicastar-Red						Unknown[(e)]		
Spherisorb		Silica gel	5,000			No[(e)]		
Ludox®	CL	Colloidal silica				No		
	AS-20					No		
	AM					No		
	SM-30					No		
Tixosil®	T43	Precipitated silica	5–15	TEM	250 ± 30	Yes		Yes
	T73		15–25	TEM	85 ± 25	No		
Levasil	200	'Amorphous silica, aqueous colloidal solution'	5–100		200	No		
	50		5–100		50	No		

BET: Brunauer–Emmett–Teller; TEM: transmission electron microscopy.
(a): Specific food-grades of AEROSIL 380 (AEROSIL 380F) and AEROSIL 200 (AEROSIL 200F) are used as food additives.
(b): Not produced anymore.
(c): Not commercial.
(d): Current name is TS720.
(e): This product is not manufactured by ASASP member companies; based on the information found in internet these product is not suitable to be used as food additive E 551.

Appendix B – Primary particle size of the engineered nano-SAS series from the JRC Repository (JRC, 2013) used in biological and toxicological studies

Name of the substance	Nature of material	Primary particle size (nm)	Primary particle size distribution	Method used to determine particle size	Specific area (BET method) (m^2/g)	Reference
NM-200	Precipitated silica	14–23	89% below 100 nm 70% below 50 nm 2% below 10 nm	TEM	189	JRC (2016)
NM-201	Precipitated silica	17–19	82% below 100 nm 55% below 50 nm 1% below 10 nm		140	
NM-202	Fumed silica	15–20	80%% below 100 nm 55% below 50 nm 1% below 10 nm		204	
NM-203	Fumed silica	13–45	78% below 100 nm 48% below 50 nm	TEM	203	JRC (2014)

BET: Brunauer–Emmett–Teller; TEM: transmission electron microscopy.

Appendix C – Data on the particle size of SiO_2 (E 551) from the literature

	Dekkers et al. (2011)		Peters et al. (2012)	Heroult et al. (2014)	Contado et al. (2013)	
Samples	AEROSIL 200F and AEROSIL 380F (see more information in Appendix A)	32 food products	3 food products (black coffee, soup and pancake)	1 food product (coffee creamer)	4 samples of SAS: AEROSIL300, AEROSIL380, Tixosil43 and Tixosil73 380F (see more information in Appendix A)	2 food products (a powdered 'cappuccino' mixture and a food integrator)
Analytical method(s) used	TEM	HDC-ICP-MS	HDC-ICP-MS (additional measurements with DLS and SEM)	FFF-ICP-MS	SdFFF, SEM, TEM	SdFFF, SEM
Sample preparation		Food samples were suspended in "LC" or Milli-Q water using ultrasonic liquid processor XL 2000 for 15 min. Suspension filtrated prior measurement. Some sample resuspended by a sonic bath for 15 min		The samples were defatted with hexane prior to suspension in water and applied sonication. A suspension of the crude creamer in water using mechanical shaking was also analysed	Protocol: vortexing the suspension for 30 s, ultrasonic suspension for 10 s, and vortexing the suspension for further 30 s	The samples of powdered cappuccino mix in water were dispersed with ultrasonic probe and vigorously mixed with hexane. The aqueous phase was centrifuged. The suspension of the food integrator in water was shaken with vortex and ultrasonic probe
Results	AEROSIL 200F-primary particles 12 nm (no information on the % of number of particles). AEROSIL 300F-primary particles 7 nm (no information on the % of number of particles).	Percentage of silica in nano-form range from 'not measured' in 21 food products to up to 19% (coffee creamer) or 33% (instant asparagus soup. Analysis of a freshly prepared cup of coffee containing coffee creamer (sample	Percentage (relative to total silica) of nano-silica (5–200 nm) in food products in water range from 5% to 29% (higher for black coffee)	11% of total silicon within the size of 1–100 nm	A300 (TEM): primary particles (7–10 nm) with tendency for form aggregates of different sizes. A380 (SEM): structure of aggregates with clusters of 50–200 nm. T43 (SEM): primary	Most of the silica particles were organised in aggregates and agglomerates > 100 nm; the food integrator (rich in silica) showed a more heterogeneous population of aggregates than the cappuccino mixture and presented

	Dekkers et al. (2011)		Peters et al. (2012)	Heroult et al. (2014)	Contado et al. (2013)	
	Most of the primary particles seem to form larger aggregated and/IR agglomerates	not sonicated). The percentage of silica in nano-form in the processed food (coffee with coffee creamer) was more than two times higher than from non-processed food			particles (25–50 nm) are organised in clusters of different sized forming agglomerates that exceed 10 m. T73 (SEM): irregularly shaped particles (80–100 nm), some spherical particles (200–300 nm) and a majority of larger aggregates	only a limited number of isolated particles smaller than 100 nm

	Athinarayanan et al. (2015)		Yang et al. (2016)		Contada et al. (2016)	Barahona et al. (2016)
Samples	Commercially available E 551 (no further information available)	2 food products (a commercial brand of 'zero calorie' sweetener and a commercial brand of a powdered vanilla flavour)	6 samples of food-grade SiO_2 from commercial vendors in the USA and China (no further information available)	13 food products (including tablets)	4 samples of SAS (AEROSIL300, AEROSIL380, Tixosil43 and Tixosil73 380F (see more information in Appendix A). 1 food sample (instant cappuccino mix)	11 samples of SAS (Syloid Al-1, Syloid 244, Syloid 72, Tixosil 38, Tixosil 43, Tixosil 73, AEROSIL380, Cab-O-Sil M-5F, Cab-O-Sil EH-5F, Tixosil 38 AB, Tixosil 38 A) (see more information in Appendix A)
Analytical method(s) used	DLS	TEM	TEM	TEM and SEM	DCS and SdFFF	DLS, AF4-MALS-ICP-MS, TEM
Sample preparation		Samples were disperse using sonication 30 min. The pellet was redispersed in ethanol for TEM analysis	Suspension was sonicated (30 min) in a water bath sonicator	Suspension was sonicated (30 min) in a water bath sonicator. Then, the suspension was centrifuged. Pellet was re-suspended in water	Suspension were mixed on a vortex (30 s) and then: stirred using magnetic bar (15 min); or sonicated 120 s; or sonicated in water bath for 15 min	Ultrasonic probe used as dispersing technique

	Athinarayanan et al. (2015)		Yang et al. (2016)		Contada et al. (2016)	Barahona et al. (2016)
Results	Average size 160 nm	Primary particles (20–50 nm) were aggregated	All samples contained agglomerates (0.5–2 m); the mean diameters of all primary particles below 100 nm with mean primary particle sizes of 9–26 nm (no quantification)	Nanoparticles of SAS observed in 10 out of the 13 samples (no quantification). Morphology and sizes of SAS in food samples similar to the food-grade SiO_2	The 4 samples of SAS consist of nano-primary particles, aggregates and agglomerates to build entities of large sizes. Aggregates and agglomerates break to smaller entities, whose size and relative amount depend on the dispersion methods. Cappuccino mix contains particles and/or aggregates of sizes spanning between 30 nm to 2 m	DLS: hydrodynamic diameter range 152.3–202 nm for fumed SASs and 284.9–644.6 nm for the other samples. AF4-ICPMS: in 7 out of the 11 samples particles < 100 nm (number of particles/L 10^{-11} range 0.1–11.4). TEM characterisation confirmed that all the samples contained primary particles and small aggregated below 100 nm

TEM: transmission electron microscopy; HDC: hydrodynamic chromatography; ICP-MS: inductively coupled plasma mass spectrometry; FFF: field flow fractionation; SdFFF: sedimentation field flow fractionation; SEM: scanning electron microscopy; DLS: dynamic light scattering; AF4: asymmetric flow-field flow fractionation; MALS: multiangle light scattering.

Appendix D – Summary of the reported use levels in food (mg/kg or mg/L) of silicon dioxide (E 551) provided by industry

Appendix D can be found in the online version of this output ('Supporting information' section): http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2018.5088/suppinfo/

Appendix E – Number and percentage of food products labelled with silicon dioxide (E 551) out of the total number of food products present in the Mintel GNPD per food subcategory between 2012 and 2017

Appendix E can be found in the online version of this output ('Supporting information' section): http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2018.5088/suppinfo/

Appendix F – Concentration levels of silicon dioxide (E 551) used in the exposure scenarios (mg/kg or mg/L as appropriate)

Appendix F can be found in the online version of this output ('Supporting information' section): http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2018.5088/suppinfo/

Appendix G – Summary of total estimated exposure of silicon dioxide (E 551) per population group and survey: mean and 95th percentile (mg/kg bw per day)

Appendix G can be found in the online version of this output ('Supporting information' section): http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2018.5088/suppinfo/

Appendix H – Main food categories contributing to silicon dioxide (E 551) (> 5% to the total mean exposure)

Appendix H can be found in the online version of this output ('Supporting information' section): http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2018.5088/suppinfo/

Appendix I – Genotoxicity studies

Appendix I can be found in the online version of this output ('Supporting information' section): http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2018.5088/suppinfo/