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SCIENTIFIC OPINION

Re-evaluation of silicon dioxide (E 551) as a food additive in foods for infants below 16 weeks of age and follow-up of its re-evaluation as a food additive for uses in foods for all population groups

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Abstract

The present opinion is the follow-up of the conclusions and recommendations of the Scientific Opinion on the re-evaluation of silicon dioxide (E 551) as a food additive relevant to the safety assessment for all age groups. In addition, the risk assessment of silicon dioxide (E 551) for its use in food for infants below 16 weeks of age is performed. Based on the newly available information on the characterisation of the SAS used as E 551 and following the principles of the 2021 EFSA Guidance on Particle-TR, the conventional safety assessment has been complemented with nano-specific considerations. Given the uncertainties resulting from the limitations of the database and in the absence of genotoxicity concern, the Panel considered that it is not appropriate to derive an acceptable daily intake (ADI) but applied the margin of exposure (MOE) approach for the risk assessment. The Panel concluded that the MOE should be at least 36 for not raising a safety concern. The calculated MOEs considering the dietary exposure estimates for all population groups using the refined non-brand loyal scenario, estimated at the time of the 2018 re-evaluation, were all above 36. The Panel concluded that E 551 does not raise a safety concern in all population groups at the reported uses and use levels. The use of E 551 in food for infants below 16 weeks of age in FC 13.1.1 and FC 13.1.5.1 does not raise a safety concern at the current exposure levels. The Panel also concluded that the technical data provided support an amendment of the specifications for E 551 laid down in Commission Regulation (EU) No 231/2012.

The paucity of toxicological studies with proper dispersion protocol (with the exception of the genotoxicity studies) creates uncertainty in the present assessment of the potential toxicological effects related to the exposure to E 551 nanosize aggregates.

KEYWORDS
aggregates, E 551, food additive, infants, nano risk assessment, nanoparticles, silicon dioxide

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SUMMARY

The safety of silicon dioxide (E 551) was re-evaluated by the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) under Regulation (EU) No 257/2010, as part of the re-evaluation programme for food additives authorised in the EU before 20 January 2009 (EFSA ANS Panel, 2018). In that opinion, the ANS Panel had identified data gaps and uncertainties that required follow-up by means of a call for data aimed at gathering information from interested business operators (IBOs). The information sought also included the data needed to perform the risk assessment of silicon dioxide (E 551) for its use in food for infants below 16 weeks of age (according to Annex III, Part 5, Section B of Regulation (EC) No 1333/2008), not covered by the 2018 ANS Panel opinion. The ANS Panel had issued recommendations to amend the specifications of the food additive E 551 in Commission Regulation (EU) No 231/2012, also with respect to the characterisation of particle size distribution.

The present opinion provides an updated safety assessment of the food additive E 551 for all age groups, including the safety in use in food for infants below 16 weeks of age, and taking into account all new relevant data available to EFSA since the completion of its re-evaluation in 2018.

Based on the data on different commercialised E 551 provided by interested business operators (IBOs) in response to the EFSA follow-up call, the food additive can be described as synthetic amorphous silica (SAS), without crystalline structure, not coated or surface functionalised. Different types of SAS are used as E 551: fumed (or pyrogenic) silica produced by the pyrolysis method; precipitated silica, silica gel and hydrous silica produced by the wet method. SAS consists of near-spherical nano-sized constituent particles (with the majority exhibiting D50 from 2 to 28 nm) forming complex (fractal-like) aggregates.

Considering the characterisation of SAS used as E 551 and that SAS is insoluble/very slightly soluble in water, in line with the EFSA Guidance on Particle – Technical Requirements (EFSA Scientific Committee, 2021a), E 551 requires risk assessment at the nanoscale following the EFSA Guidance on Nano – Risk Assessment (EFSA Scientific Committee, 2021b), to complement the conventional risk assessment conducted according to the applicable sectoral guidance. The data provided by IBOs in response to the call were therefore complemented with data retrieved from the published literature and considered to be relevant for the assessment at the nanoscale, in line with the data requirements specified in the 2021 'EFSA Guidance on Nano – Risk Assessment' as well as additional unpublished studies conducted in the frame of the Nanogenotox project. All the evidence was appraised for their relevance and reliability according to predefined criteria described in the opinion.

The support of the EFSA cross-cutting Working Group (cc WG) genotoxicity was requested to review the evidence and conclude on the genotoxicity of E 551. The EFSA cc WG on nanotechnologies provided advice to the Panel with respect to criteria to be used for the appraisal of specific nanoscale considerations (NSC), relevant for the investigation of the potential toxicological, including genotoxic, effects due to the nano-sized particles/aggregates of E 551. This appraisal step led to the grading of the studies into four categories, ranging from 1 to 4, from the highest level of confidence that nano-sized particles/aggregates were tested under the experimental conditions (NSC 1), to the lowest (NSC 4).

The toxicokinetics of E 551 have been investigated in vitro and in vivo studies. After endocytotic uptake, E 551 is present in the cells of different organs where it is dissolved to silicic acid ($Si(OH)_4$). Silicic acid is partly dissociated and silicic acid and Si in ionic form are excreted by the kidneys by glomerular filtration.

In the in vitro studies, endocytotic uptake mechanism of SAS in cells and its intracellular distribution was demonstrated qualitatively. Qualitative data on particles localised in several organs were available only in a small number of in vivo studies. Despite the uncertainties in the toxicokinetics data, the Panel nonetheless estimated that very low systemic availability is expected, although this has not been adequately quantified. The data also showed that the relative systemic availability, when expressed as a percentage of the dose, decreased with increasing dose, probably because of a saturated uptake process.

Based on the currently available data set, the Panel concluded that E 551 does not raise a concern for genotoxicity. The pattern of positive and negative results does not change significantly when considering the results all together for all the available studies (NSC 1–4) or separately for NSC 1–2. Therefore, the Panel considered that the conclusion on the genotoxicity is applicable to nano aggregates present in E 551.

The Panel performed an overall weight of evidence (WoE) analysis by grouping the toxicological studies according to the target organ/system investigated and the related endpoints. In a first step, all studies were included in the WoE independently from their NSC score. In a second step, an analysis was performed comparing the outcome of the studies with NSC 1 and 2 and of the studies with NSC 3 or 4, to verify whether there could be specific findings related to the exposure to nano-sized particles/aggregates compared to larger aggregates/agglomerates.

No study reported adverse effects on general toxicity-relevant endpoints (mortality, body weight, appearance and behaviour, clinical signs, haematology, functional observations, blood electrolytes) up to the highest dose tested of 5000 mg SAS/kg body weight (bw) per day. Liver and spleen are the two organs with the highest Si concentrations and content after administration of SAS. For both organs, no adverse effects were noted concerning the investigated parameters (weight, histopathology and liver enzymes) up to the highest dose tested of 5000 mg SAS/kg bw per day. Based on the WoE, the Panel considered it likely that E 551 has no adverse effects on general toxicity relevant endpoints, on liver and spleen.

No adverse effects were found on other endpoints including weight and histopathology of the kidney, creatinine and urea serum levels, urinary tract histopathology up to the highest dose tested of 5000 mg/kg bw per day. No adverse effects were found in gastrointestinal tract, lungs, bones, endogenous metabolism parameters at oral doses up to or exceeding

1000 mg SAS/kg bw per day. Based on the WoE, the Panel considered it likely that E 551 has no adverse effects on the above-mentioned parameters.

No adverse effects were found on adrenals weight or histopathology, thyroid/parathyroids or pituitary weight and histopathology, or on thyroid hormones at oral doses up to or exceeding 1000 mg SAS/kg bw per day. Based on the WoE, the Panel considered it likely that E 551 has no adverse effects on these organs and hormones.

In the weight of evidence analysis for reproductive and developmental toxicity, no adverse effects were reported at oral doses up to or exceeding 1000 mg SAS/kg bw per day. No adverse effects were reported at oral doses up to or exceeding 1000 mg SAS/kg bw per day in neurotoxicity-relevant endpoints (brain weight and histopathology). Based on the WoE, the Panel considered it likely that E 551 has no adverse effects on reproduction and development and no adverse effects on the nervous system.

With respect to the immunotoxic potential of SAS. Various parameters related to immune system status were investigated across 12 studies considered for the assessment. These included blood leucocytes, lymphocyte subpopulations, cytokine production and immune-mediated disorders. Due to the limited number of studies, differing doses and inconsistent findings, the Panel considered that although some findings might suggest immunotoxicity, related apical effects were not consistently reported. Based on the currently available data, the Panel concluded that it is likely that E 551 has no adverse effects on the immune system. However, the ability of SAS to modify intestinal immune homeostasis by inducing low-grade intestinal inflammation, which might predispose to food allergy and autoimmune-mediated coeliac diseases, deserves further investigation, particularly in humans.

Based on the overall WoE analysis, the Panel considered the available data set of in vivo studies as suitable to assess toxicological potential of large agglomerates, and of agglomerates and aggregates in the nanosize range, with uncertainty on the degree of agglomeration. Only two studies (addressing general toxicity endpoints, liver, kidney, adrenals, immune, gastrointestinal tract, vascular toxicity) using low doses and of short duration were available to assess the hazard of nanosized aggregates. Therefore, the Panel considered that the studies included in the WoE analysis can inform on the potential toxicity of nanosize aggregates/agglomerates only to a limited extent.

The Panel also acknowledged that there is still uncertainty on the size/morphology of the aggregates for the different SAS used as E 551. No information on the relative amounts of particles occurring as isolated particles, aggregates and/or as agglomerates in food is available, and no quantitative information on the level of aggregation/agglomeration of E 551 in food is known. Other uncertainties were identified in the available data set.

In its 2018 opinion, the ANS Panel had considered that it would have been possible to derive an ADI for the food additive E 551 should the limitations in the toxicological data set be reduced. Hence, in the absence of a genotoxicity concern, the Panel considered which approach would be most appropriate to assess the safety of E 551 taking into account that no adverse effect was seen in all included animal studies up to a dose of 5000 mg/kg bw per day albeit with uncertainty on the testing of small aggregates in the nanosize range. Given the remaining uncertainties resulting from the limitations of the available database, the FAF Panel considered that it was still not appropriate to derive an ADI for this food additive and decided to apply the margin of exposure (MOE) approach to evaluate the safety of E 551.

For the identification of a reference point (RP), the Panel noted that, from the available toxicity data set, the highest dose tested without adverse effects was 1000 mg E 551/kg bw per day in the chronic study in rats and 2047 mg E 551/kg bw per day in the 84-day study in rats. The latter dose of 2047 mg E 551/kg bw per day was selected by the Panel as the relevant RP despite being identified from the study of shorter duration because the Panel considered that in the 84-day study, nanoparticle characteristics had been taken into account better than in the 1-year study.

Specific toxicokinetic considerations were applied to the RP identified above allowing a reduction of the required MOE to 36 instead of the default value of 100.

In order to calculate the resulting MOE, the Panel considered appropriate to refer to the dietary exposure assessment already performed by the ANS Panel at the time of the 2018 re-evaluation and reconfirmed the choice of the refined, non-brand loyal scenario as the most appropriate and realistic scenario for risk characterisation of E 551 for the general population (above 16 weeks of age). The Panel noted that the resulting MOEs were above 36 for all age groups, indicating that there would be no safety concern at the reported use and use levels.

In addition to the conventional risk assessment based on external doses, the Panel decided to complement its assessment by estimating margin of internal exposure. To do so, the existing data were used to compare the mean Si concentration in organs in humans to the highest Si concentration in the same organs in animal toxicity studies without effects. Comparing the average liver Si concentration in humans of 8 mg Si/kg with the liver Si concentration in experimental animals of 100 mg/kg, the resulting margin of internal exposure is 12.5. Comparing the average spleen Si concentration in humans of 12 mg Si/kg with the spleen Si concentration in experimental animals of 110 mg/kg, the margin of internal exposure is 10. For the MOE calculation, the default value for the toxicodynamic interspecies difference and the intraspecies variability is 8 (2.5×3.2), because inter- and intra-species toxicokinetic differences do not have to be considered when comparing internal concentrations, and only toxicodynamic differences are relevant. The Panel considered that this additional analysis supports the conclusion of no safety concern for E 551 at the reported use and use levels for the general population.

At the time of the 2018 ANS Panel re-evaluation opinion, the safety of E 551 for infants below 16 weeks of age had not been addressed, and therefore, the safety of the use of E 551 in foods intended for this population group is covered in the present updated assessment, performed in accordance with the applicable 2017 EFSA Guidance of the Scientific Committee on the risk assessment of substances present in food intended for infants below 16 weeks of age.

The exposure to E 551 in this age group was estimated at the maximum use levels as 3 mg/kg bw per day for the high consumption in both infant formulae (FC 13.1.1) and in special infant formulae for medical purposes (FC 13.1.5.1). The available hazard data set did not include any human or animal data on this age group. In the absence of this information, and in order to assess the safety in this age group the Panel considered the reported adverse events potentially related to simeticone, a medicinal product containing SAS, widely used for more than 40 years in infants. Following this approach, the Panel considered that there is no safety concern of SAS as an active ingredient of simeticone up to a dose of 2.37 mg/kg bw per day, the therapeutic dose in the relevant age group. Because the maximum highest exposure estimates (3 mg/kg bw per day) were comparable to the level without adverse side effects seen for simeticone and, taking into account that no adverse effects were seen in animal studies at doses up to 5000 mg/kg bw per day, the Panel considered that there is no safety concern for the use of E 551 as food additive according to Annex III to Regulation (EC) no 1333/2008 in food intended for infants below 16 weeks of age at the current exposure levels.

Overall, the Panel concluded that E 551 does not raise a safety concern in all population groups at the reported uses and use levels.

The Panel also concluded that the technical data provided support an amendment of the specifications for silicon dioxide (E 551) laid down in Commission Regulation (EU) No 231/2012, and recommended lowering the maximum limits for lead, mercury and arsenic and introducing a maximum limit for aluminium.

1 | INTRODUCTION

The re-evaluation of silicon dioxide (E 551) as a food additive was completed by EFSA in 2017 (EFSA ANS Panel, 2018). The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) issued several recommendations to amend the specifications of the food additive E 551 in Commission Regulation (EU) No 231/2012,[1] also with respect to the characterisation of particle size distribution.

The data gaps and uncertainties identified by the ANS Panel required a follow-up by EFSA by means of a call for additional data.[2]

The present opinion deals with:

- The follow-up on issues that have been expressed in the conclusions and recommendations of the Scientific Opinion on the re-evaluation of silicon dioxide (E 551) as a food additive (EFSA ANS Panel, 2018), and relevant to the safety assessment for all age groups.
- The risk assessment of silicon dioxide (E 551) for its use in food for infants below 16 weeks of age, according to Annex III, Part 5, Section B of Regulation (EC) No 1333/2008.[3]

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

1.1.1 | Background

The composition of food intended for infants and young children, as defined by Regulation (EU) No 609/2013,[4] is regulated at EU level and such rules include requirements concerning the use of substances as food additives.

The use of food additives is regulated by Regulation (EC) No 1333/2008 on food additives. Only food additives that are included in the Union list, in particular in Annex II and III to that Regulation, may be placed on the market and used in food under the conditions of use specified therein.

In accordance with Regulation (EU) No 257/2010,[5] EFSA is currently re-evaluating the safety of food additives already permitted in the Union before 20 January 2009 and issuing scientific opinions on their safety when used in food as per Annexes II and III to Regulation (EC) No 1333/2008. However, the risk assessment approach followed until now has not covered the use of food additives in food for infants below 12 weeks of age. Consequently, EFSA published several scientific opinions on the re-evaluation of the safety of food additives permitted in food category 13.1 but not addressing their use in food for infants below 12 weeks of age.

In addition, in these opinions EFSA identified some concerns, namely (1) Data gaps that have triggered recommendations in the (to be) published scientific opinions, and/or; (2) Data gaps that have increased uncertainties linked to the risk assessment and/or which prevented the EFSA from concluding on some aspects of it.

[1]Commission Regulation (EU) No 231/2012: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32012R0231.

[2]Call for technical and toxicological data on silicon dioxide (E 551) for uses as food additive in foods for all population groups including infants below 16 weeks of age. Published on 10 October 2018. https://www.efsa.europa.eu/en/consultations/call/call-technical-and-toxicological-data-silicon-dioxide-e-551-uses.

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

[4]Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control. OJ L 181, 29.6.2013, p. 35–56.

[5]Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a program 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.

On 31 May 2017, EFSA published a guidance document (EFSA Scientific Committee, 2017) on the risk assessment of substances present in food intended for infants below 16 weeks of age, thus enabling EFSA to assess the safety of food additives used in food for infants below 12 weeks of age.[6] Now EFSA is expected to launch dedicated calls for data to be able to perform such risk assessments.

The EC considers it is more effective that EFSA, in the context of these dedicated calls for data, also addresses all the issues and data gaps already identified in the relevant (to be) published scientific opinions on the re-evaluation of the safety of food additives permitted in food category 13.1.

In accordance with the current EC approach for the follow-up of EFSA's scientific opinions on the re-evaluation of the safety of permitted food additives for which some concerns have been identified, a specific call for data would be published by the EC on DG SANTE's website[7] on food additives and additional (missing) information would then be provided by interested business operators to the EC.

However, for those scientific opinions on the re-evaluation of the safety of permitted food additives in food category 13.1 for which the risk assessment does not address their uses in food for infants below 12 weeks of age and for which some concerns have been identified by EFSA, the EC considers that for the sake of efficiency it would be appropriate to streamline the approach as described above.

Therefore, the EC requests EFSA to address all the issues and data gaps already identified in the relevant published scientific opinions of those food additives (or groups of additives that can be addressed simultaneously) as part of the upcoming work on the safety assessment of food additives uses in food for infants below 12 weeks of age.

This follow-up aims at completing the re-evaluation of the food additives in question for all food categories and includes calls for data covering the actual use and usage levels of food additives in food for both infants below 12 or 16 weeks of age as well as for older infants, young children and other groups of the population for which EFSA has already finalised its assessment.

The future evaluations of EFSA should systematically address the safety of use of food additives for all age groups, including the infants below 12 or 16 weeks of age.

1.1.2 | Terms of Reference

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002,[8] and as part of EFSA's work in completing its risk assessments concerning the use of food additives in food for infants below 12 weeks of age, covered by the re-evaluation programme and its terms of reference, the European Commission requests the European Food Safety Authority to address all the data gaps specified in the recommendations made in its scientific opinions on the re-evaluation of the safety of food additives permitted in food category 13.1 (food for infants and young children) of annex II to Regulation (EC) No 1333/2008.

1.1.3 | Interpretation of Terms of Reference

Before the publication of the EFSA Scientific Committee Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age (EFSA Scientific Committee, 2017), EFSA has taken 12 weeks as a cut off age for the applicability of the safety assessment. However, according to EFSA Scientific Committee (2017), the assessment will include infants up to 16 weeks of age because they are exposed to formula feeding until this age as the only source of food since complementary feeding is not supposed to be introduced before this age (see EFSA Scientific Committee, 2017).

The data provided by interested business operators (IBOs) in response to the follow-up call issued after the 2018 ANS Panel opinion show that the constituent particles[9] in synthetic amorphous silica (SAS) used as E 551 are in the nanosize range with the majority exhibiting median minimal external dimension values from 2 to 28 nm (see Section 3.2.3 of this Opinion). SAS is insoluble/very slightly soluble in water (SCCS, 2019) (see Section 3.1). Therefore, in line with the EFSA Guidance on Particle – Technical Requirements (EFSA Scientific Committee, 2021a), E 551 requires risk assessment at the nanoscale following the EFSA Guidance on Nano – Risk Assessment (EFSA Scientific Committee, 2021b), to complement the conventional risk assessment conducted according to the applicable sectoral guidance (see Section 2.2).

1.2 | Summary of the EFSA re-evaluation of silicon dioxide (E 551) as a food additive under Regulation (EU) No 257/2010

In 2017 the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) provided a scientific opinion re-evaluating the safety of silicon dioxide (E 551) when used as a food additive (EFSA ANS Panel, 2018) under Regulation (EU)

[6]See Section 1.1.3.

[7]https://ec.europa.eu/food/safety/food_improvement_agents/additives/re-evaluation_en.

[8]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

[9]Also called primary particles in ISO 26824:2022. https://www.iso.org/obp/ui/#iso:std:iso:26824:ed-2:v1:en.

No 257/2010. The ANS Panel had noted that forms of synthetic amorphous silica (SAS) used as E 551 include fumed silica and hydrated silica (precipitated silica, silica gel and hydrous silica). 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. The ANS Panel concluded that the EU specifications were insufficient to adequately characterise the food additive E 551. Clear characterisation of particle size distribution was required. Although silicon dioxide appeared to be poorly absorbed, silicon-containing material (in some cases presumed to be silicon dioxide) was found in some tissues. Despite the limitations in the sub-chronic, reproductive and developmental toxicological studies, including studies with nano silicon dioxide, there was no indication of adverse effects. E 551 did not raise a concern with respect to genotoxicity. In the absence of a long-term study with nano silicon dioxide, the ANS Panel could not extrapolate the results from the available chronic study covering the full range of nanoparticle sizes that could be present in the food additive E 551. The highest population exposure estimates were at least one order of magnitude lower than the no observed adverse effect levels (NOAELs) identified in the animal studies (the highest doses tested). Based on the available database, there was no indication for toxicity of E 551 at the reported uses and use levels in consumers. Because of the limitations in the available database, the ANS Panel could not confirm the ADI 'not specified' set in 1991 by the Scientific Committee on Food (SCF) (SCF, 1991), and recommended that the limitations of the toxicological database should be reduced. The ANS Panel recommended to the EU Commission that the specifications for E 551 should be modified.

2 | DATA AND METHODOLOGIES

2.1 | Data

The Panel based its assessment on the following information:

- Information submitted by IBOs in response to the EFSA public call for data and subsequent requests for clarifications and/or additional information (Documentation provided to EFSA n. 1–35),
- The conclusions and recommendations from previous evaluation (EFSA ANS Panel, 2018),
- Information from Mintel's Global New Products Database (GNPD) to identify the use of the food additive silicon dioxide (E 551) in food products for infants below 16 weeks of age,
- Additional information was retrieved from a literature search. For further details on the literature search, please refer to the section 'Methodologies'.
- Available study summaries of the genotoxicity studies conducted in the frame of Nanogenotox project and reported in the OECD report No. 71, 2016 (OECD, 2016).

2.2 | Methodologies

This opinion was formulated following the principles described in the EFSA Guidance of the Scientific Committee 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 including the EFSA Guidance of the Scientific Committee on the risk assessment of substances present in food intended for infants below 16 weeks of age (EFSA Scientific Committee, 2017) and the Scientific Committee Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: human and animal health (EFSA Scientific Committee, 2021b).

Terms and definitions related to nanomaterials in this document are used as defined by the European Commission's Joint Research Centre (Rauscher et al., 2023).

The current assessment addressed whether dietary exposure to the food additive E 551 in its reported uses and use levels in food intended for all population groups, including infants below 16 weeks of age, raise a health concern. In the light of the characterisation of the food additive E 551 (see Section 3.4), special attention was given to the nanoscale considerations.

A literature search was performed following the approach described in Annex A. The bibliographic databases PubMed and Web of Science Core Collection were searched to identify relevant studies.

The search strings, as run in the sources of information, have been structured to include the combination of silicon dioxide identifiers and terms related to toxicity outcomes or types of studies and study design relevant to assess the questions of interest. The searches have been limited to documents published in the English language from the year 2000 onwards. Limits to remove editorial material and letters from the resulting literature have been applied. The cut-off year of 2000 was selected based on the scientific knowledge in the areas of nanomaterials.

More specifically, searches were run to cover a period from 2000 to 1st March 2024: 25,235 references were retrieved by EFSA and imported into DistillerSR software for screening. An initial screening of all references by titles and abstracts based on the inclusion and exclusion criteria was performed (see Appendix A, Criteria for inclusion/exclusion applied to literature publications). Each reference was screened independently by two external contractor reviewers. A total number of included 985 references, preliminarily identified as possibly relevant, were further screened at full text level for the relevance of the tested material as food additive and classified according to the type of toxicity study (see Appendix B, Criteria

for classification of the toxicological studies applied to the literature search). As result of this initial step, references were excluded from further assessment if conducted with test material not relevant for E 551 and with routes of exposure or test systems deemed non relevant. The criteria for inclusion and exclusion were defined as indicated in the Appendix A. The advice from the EFSA ccWG Nanotechnology was considered in defining these criteria (Appendix C, Advice of the cross-cutting WG on Nanotechnologies on nanoscale considerations for the assessment of the study design and study results of SAS toxicity studies).

Toxicity studies were considered for the assessment of E 551 if the test material was:

(i) the food additive silicon dioxide (E 551),
(ii) SAS material from the JRC repository (NM-200, NM-202, NM-203 and NM-204), which IBOs have indicated are representative of E 551 (Documentation provided to EFSA No. 7; Rasmussen et al., 2013),
(iii) SAS used in studies described in peer-reviewed publications for which the information in the publication regarding the physicochemical characterisation of the test item is limited, but indicates that the material consists of near-spherical nano-sized constituent particles forming complex (fractal-like) aggregates in line with the proposed definition of E 551 in the EU specifications (Section 3.4),
(iv) NM-201 or SAS which are not used as E 551, but comply with the proposed definition of E 551 as indicated in (iii).

Toxicity studies performed with the materials under point (iii) and (iv) have been considered in order to identify whether specific information or restrictions should be included in the proposed amendment of the EU specifications for E 551.

In addition to the references retrieved in the literature search, the studies evaluated in the re-evaluation have been screened for inclusion on the basis of the test material used; only studies conducted with a relevant test material were included as well as publications from year 2000. Moreover, studies provided by the IBO (Documentation provided to EFSA No. 1; 4; 10-21; 24-26; 29; 31-34) and the available study summaries of the genotoxicity studies conducted in the frame of Nanogenotox project and reported in the OECD report No. 71 (2016) have been included. These studies were screened based on the same criteria applied to the references identified from the literature search.

A total of 205 references (from the literature and provided by the IBO or other parties) passed the second screening step.

The support of the EFSA cc WG Genotoxicity was requested to review the evidence and conclude on the genotoxicity of E 551. The 59 genotoxicity references have been assessed by the EFSA ccWG Genotoxicity based on their approach for assessing reliability and relevance of genotoxicity studies (EFSA, 2023). This assessment (referred to in Section 3.5.4 and Annex B) was submitted to the Panel for its consideration and decision.

Regarding toxicity studies other than genotoxicity, 146 references have been assessed independently by two members of the FAF WG Follow-up Tox for relevance and reliability. Divergent views were resolved by discussions among the WG members.

The Panel agreed to systematically assess all the relevant and reliable toxicity studies conducted in vivo that could contribute to the identification and characterisation of potential adverse effects associated with dietary exposure to E 551. The Panel agreed to use information from in vitro studies, other than those reporting genotoxicity endpoints, only where appropriate, in order to inform on the mode(s) of action (MoA(s)) and to assess the biological plausibility of the effects observed.

Assessment of the relevance (external validity)

Studies were considered relevant if designed in such a way that they would allow (i) their use for identifying a reference point (RP) and (ii) to support the risk assessment (see Appendix D, Approach for the appraisal of the toxicity studies).

Assessment of the internal validity

In vivo studies that were confirmed as relevant were individually appraised for their reliability based on the OHAT Risk of Bias (RoB) Rating Tool for Human and Animal Studies (OHAT, 2015), which has been modified and used for the appraisal of both animal and human studies (see Appendix D). This appraisal step led to the grading of the available studies into four categories, ranging from 1 to 4, from the highest level of internal validity (RoB 1), indicating that the design and conduct of the study allow to evaluate the association (or the lack of it) between the exposure to the test material and the reported outcome, to the lowest (RoB 4) (see Appendix D). The rating was based on experts' judgement, with a narrative description of the elements considered for the overall rating. The ADME and acellular degradation studies have been evaluated by the experts narratively since the applied scoring elements from OHAT related to the aspects of the design and performance of the studies were not applicable for such studies.

Only toxicological studies considered of sufficient reliability based on the internal validity grade (RoB 1 and 2) were included in the assessment. Studies with RoB 3 have been used as supporting evidence in selected cases with a justification. Studies of insufficient internal validity (RoB 4) were not considered in the assessment. The studies rated as RoB 3 (not used as supporting evidence) and RoB 4 have been listed in tabular format in Annex C alongside a justification for reliability scoring by bias domain.

Nanoscale considerations

The data provided by IBOs show that the constituent particles[10] in SAS used as E 551 are in the nanosize range, with the majority exhibiting median minimal external dimension values from 2 to 28 nm (see Section 3.2.3 of this Opinion). SAS is insoluble/very slightly soluble in water (SCCS, 2019) (see Section 3.1). Therefore, in line with the EFSA Guidance on Particle – Technical Requirements (EFSA Scientific Committee, 2021a), E 551 requires risk assessment at the nanoscale following the EFSA Guidance on Nano – Risk Assessment (EFSA Scientific Committee, 2021b), to complement the conventional risk assessment conducted according to the applicable sectoral guidance.

The Panel noted that the principles of the EFSA Guidance on Nano – Risk Assessment recommend that toxicity testing must be performed applying protocols that would ensure a high level of dispersion of E 551 in toxicological studies. This is considered to cover the worst-case exposure scenario (EFSA Scientific Committee, 2021b). Applying such conditions would facilitate potentially higher levels of gastrointestinal absorption in in vivo studies and/or of cellular uptake in in vitro studies. The ccWG on Nanotechnologies provided the Panel an advice to guide the appraisal of the confidence that a given experimental study was appropriate to investigate the toxicological potential including genotoxic effects due to the nano-sized particles/aggregates of E 551 (Appendix C). The appraisal of the nanoscale considerations (NSC) was done for all in vivo toxicokinetic studies, and for toxicological studies considered of sufficient internal validity (RoB 1 and 2) and for studies of lower internal validity (RoB 3) considered as supportive evidence. The nanoscale considerations have been taken into account also for genotoxicity studies considered as 'high' or 'limited' relevance of the results.

This appraisal step led to the grading of the studies into four categories, ranging from 1 to 4, from the highest level of confidence that nano-sized particles/aggregates were tested under the experimental conditions (NSC 1), to the lowest (NSC 4).

The Panel acknowledged that studies with the lowest score with respect to the nano-specific aspects (NSC 4) will lead to a low level of confidence that the nano-sized particles/aggregates were tested. The studies NSC 3 have been considered by the Panel in the overall weight of evidence (WoE) acknowledging the uncertainty whether the nano-sized particles/aggregates of E 551 were tested in those toxicity studies. In addition, because there is an uncertainty on the size of the aggregates/agglomerates of E 551 in food, also studies NSC 4 were considered in the WoE, since these studies would still capture potential toxic effects of larger aggregates/agglomerates.

Weighting the body of evidence

In order to perform the WoE, the Panel grouped the studies according to the target organ/system and the related endpoints (see Annex D). *In a first step*, all studies were included in the WoE independently from their nanoscale consideration score (NSC).

In a second step, an analysis was performed comparing the outcome of the studies with NSC 1 and 2 and of the studies with NSC 3 or 4, to verify whether there could be specific findings related to the exposure to nano-sized particles/aggregates compared to larger aggregates/agglomerates.

Calculation of external dose

In animal studies, when the test substance is administered in the feed or in the drinking water, but doses are not explicitly reported by the authors as mg/kg bw per day based on actual feed or water consumption, the daily intake is calculated by the Panel using the relevant default values. In case of rodents, the values as indicated in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012) are applied. In these cases, the dose was expressed as 'equivalent to mg/kg bw per day'. If a concentration in feed or drinking water was reported and the dose in mg/kg bw per day was calculated (by the authors of the study report or the Panel) based on these reported concentrations and on reported consumption data for feed or drinking water, the dose was expressed as 'equal to mg/kg bw per day'.

Dietary exposure assessment

Dietary exposure to silicon dioxide (E 551) for the general population was estimated at the time of the re-evaluation in 2017 (EFSA ANS Panel, 2018). Considering that (i) no new occurrence data are available to EFSA since 2018, and (ii) that dietary surveys from the EFSA comprehensive database giving the highest exposure estimates in the 2017 re-evaluation are still in the consumption database, the Panel considered it appropriate to refer to the dietary exposure assessment previously performed by the ANS Panel (see Table 6 in ANS Panel 2018).

Dietary exposure to silicon dioxide (E 551) from its use as a food additive in foods for infants below 16 weeks of age was estimated combining the mean and high-level consumption values for infant formulae reported for 14–27 days of life which correspond, respectively, to 200 and 260 mL/kg bw per day (EFSA Scientific Committee, 2017), with the use levels provided by the interested business operators. Different scenarios were used to calculate dietary exposure (see Section 3.3.2). Uncertainties on the exposure assessment were identified and discussed.

[10]Also called primary particles in ISO 26824:2022. https://www.iso.org/obp/ui/#iso:std:iso:26824:ed-2:v1:en.

3 | ASSESSMENT

3.1 | Identity and specifications of silicon dioxide (E 551)

According to Commission Regulation (EU) No 231/2012, the food additive silicon dioxide (E 551) is synthetic amorphous silica (SAS), which is produced by either a vapour-phase hydrolysis process, yielding fumed silica, or by a wet process, yielding precipitated silica, silica gel or hydrous silica.

The specifications for silicon dioxide (E 551) as defined in the Commission Regulation (EU) No 231/2012 and by JECFA (2017) are listed in Table 1.

TABLE 1 Specifications for silicon dioxide (E 551) according to Commission Regulation (EU) No 231/2012 and JECFA (2017).

	Commission Regulation (EU) No 231/2012	JECFA (2017)
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 thermal process, yielding pyrogenic (fumed) silica, or by a wet process, yielding hydrated silica, precipitated silica and silica gel. Pyrogenic silica is produced in an essentially anhydrous state, whereas the wet process products are obtained as hydrates or contain surface adsorbed water
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 Hydrated silica (Precipitated silica and silica gel): Not less than 94% of SiO_2 on the ignited basis
Description	White, fluffy powder or granules. Hygroscopic	Pyrogenic (fumed) silica: A pyrogenic silicon dioxide occurring as a fine, white amorphous power or granules Hydrated silica (Precipitated silica and silica gel): A precipitated, hydrated silicon dioxide occurring as a fine, white, amorphous powder or as beads or granules
Identification		
Test for silica	Positive	Passes test
Solubility	–	Insoluble in water and insoluble in ethanol (Information required)
pH		Pyrogenic (fumed) silica: 3.0–5.0 (5% slurry, 20°) Hydrated silica (precipitated silica and silica gel): 4.0–9.0 (5% slurry, 20°)
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 (fumed) silica: Not more than 2.5% (105°C, 2 h) Hydrated silica (Precipitated silica and silica gel): Not more than 8% (105°C, 2 h)
Loss on ignition	Not more than 2.5% after drying (1000°C, fumed silica) Not more than 8.5% after drying (1000°C, hydrated forms)	Pyrogenic (fumed) silica: Not more than 2.5% (1000°C, 1 h, on dried sample) Hydrated silica (Precipitated silica and silica gel): Not more than 8.5% (1000°C, 1 h, on dried sample)
Soluble ionisable salts	Not more than 5.0% (as Na_2SO_4)	–
Arsenic	Not more than 3 mg/kg	Not more than 1 mg/kg
Lead	Not more than 5 mg/kg	Not more than 3 mg/kg
Mercury	Not more than 1 mg/kg	–

Solubility

The dissolution of the SAS particle surface requires the alkaline hydroxyl ion (OH-) as a catalyst, forming monomeric silicic acid. It has been noticed that the reaction is completely reversible and that SAS is deposited on the SAS surface by the same reaction. The dissolution in water is a hydrolytic depolymerisation and the solubility of SAS is the concentration of silicic acid (Si(OH)4) monomer or dimer reached at a steady state in the depolymerisation/polymerisation equilibrium. It is reported that dissolution rate below pH 3 is very low because the concentration of OH-, acting as a catalyst, is very low. Between pH 3 and 6, the dissolution rate is proportional to the hydroxyl ion concentration (SCCS, 2019) (Figure 1).

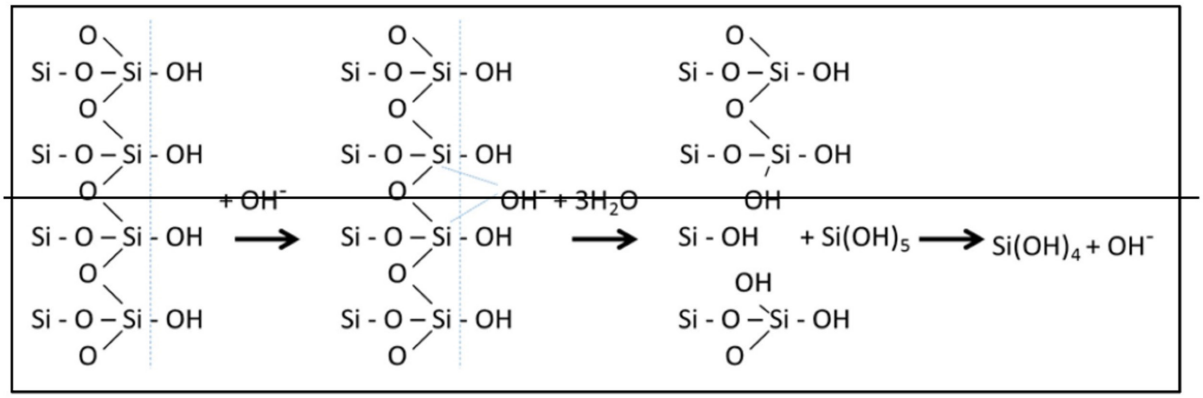

FIGURE 1 Proposed mechanism of dissolution of SAS in water in the presence of hydroxyl ions (SCCS, 2019).

Based on the information available in the SCCS (2019), SAS used as E 551 is expected to be insoluble/very slightly soluble in water.

3.2 | Technical data submitted

The following was requested in the EFSA call for data[2]:

- Particle size and particle size distribution of the food additive silicon dioxide (E 551). Because of their potential importance in toxicokinetic and toxicological effects, particle size and particle size distribution should be included in the EU specifications for the silicon dioxide (E 551) in Commission Regulation (EU) No 231/2012. Detailed and comprehensive proposed specifications for the characterisation of the fraction of nanoparticles present in the food additive silicon dioxide (E 551) should be submitted. Information on particle size and particle size distribution for the food additive silicon dioxide (E 551) supported by analytical data, in line with the 'EFSA guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health' (EFSA Scientific Committee, 2021b), is requested. This should allow the establishment of parameters in the EU specifications for silicon dioxide (E 551) that fully characterise the material used as a food additive;
- Analytical data on current levels of arsenic, lead and mercury;
- The lowest technologically achievable level for lead, mercury and arsenic in order to adequately define their maximum limits in the specifications;
- The lowest technologically achievable level for residual solvents which can be used when manufacturing silicon dioxide (E 551).

Data on different commercialised products of E 551 have been submitted to EFSA by an IBO on behalf of the Association of synthetic amorphous silica producers (ASASP).

3.2.1 | Characterisation of silicon dioxide used as E 551

According to the IBO, different types of SAS – fumed (pyrogenic) silica, precipitated silica, silica gel and hydrous silica – are used as the food additive E 551 and information on around 70 different commercialised products of E 551 was submitted (Documentation provided to EFSA No. 5 and 9).

Fumed (pyrogenic) SAS is produced by hydrolysis of volatile chlorosilanes (e.g. tetrachlorosilanes) in an oxygen (air)/hydrogen gas flame reactor. Precursor particles grow by nucleation, condensation and coagulation to yield primary[11] particles. Under the reaction conditions, primary particles collide, and stable non-dispersible aggregates are formed. By physical attraction forces (van der Waals forces and H-bridges) between aggregates, larger agglomerates are formed inside the cooling system. The solid particles are separated from the off-gas (containing hydrochloric acid) by separation methods such as filtering. Afterwards, the adsorbed hydrochloric acid on the surface of the SAS is removed by a de-acidification step (Documentation provided to EFSA No. 5).

Precipitated SAS is produced via a wet production route, by the precipitation of diluted aqueous alkali metal silicates, e.g. a water glass solution (sodium trisilicate solution), with diluted acid, e.g. sulfuric acid or hydrochloric acid in water, typically at an alkaline pH range and high temperatures. The acid and alkali metal silicate solutions are fed simultaneously into water in a stirred vessel to form silica seeds. During precipitation, three-dimensional silica primary structures are formed,

[11]The Panel considered that the terminology of primary particles used by the IBO refers to constituent particles in line with the terminology recommended by JRC (Rauscher et al., 2023).

accompanied by an increase in viscosity. The primary structures are reinforced by further precipitation of oligomeric silica and grow further into aggregates with a decrease in viscosity. The aggregates flocculate and during this process, the aggregate structure is further strengthened as the shoulders between the primary structures are filled up by SiO_2. The separation of the SAS from the reaction mixture and the washing out of the salts contained in the precipitate silica is carried out in filter aggregates such as rotary filters, belt filters or filter presses (Documentation provided to EFSA No. 5).

SAS **gel** is produced via a wet production route, by the neutralisation of diluted aqueous alkali metal silicates, e.g. a water glass solution, with a diluted acid, typically sulfuric acid. The neutralisation of sodium silicate with the mineral acid, initiates a further polymerisation of silicate tetrahedra in a random, amorphous manner to form small spheroids called primary structures. The solution containing the primary structures while still liquid is known as a hydrosol. The silica gel formation occurs when the interaction of separate micelles through hydrogen bonding, and eventual interparticle condensation, becomes significant. The gelation rate depends on many variables, such as SiO_2 concentration, pH, temperature and mixing. Polymerisation and cross-linking continue after the hydrosol has solidified into a hydrogel. The primary structure, which is the first, but unstable, solid structure, consists of SiO_2 in its interior and Si-OH on its surface. The primary structure size and the pore volumes determine the specific surface area of the silica gel (Documentation provided to EFSA No. 5).

Hydrous SAS is produced under the same conditions (wet process) as silica gel. Hydrous SAS is a less dried variant of silica gel, resulting in a higher loss on drying in comparison to the other types of SAS (see Table 1) (Documentation provided to EFSA No. 9).

According to the IBO, no components or functional groups (e.g. carboxy, amino) are inserted onto the surface of SAS used as E 551. Therefore, the surface characteristics of fumed silica and hydrated silica are attributed to their surface silanol groups (≡Si-OH), which render the particles hydrophilic (Documentation provided to EFSA No. 8).

3.2.2 | Impurities

Analytical data on the presence of toxic elements in 51 different commercial products of E 551 (7 fumed SAS, 33 precipitated SAS and 11 SAS gel) were submitted. Five samples of each product, but occasionally fewer, were analysed either after partial digestion (HNO_3), full digestion (HF) or directly on solid samples without a preliminary digestion step (Documentation provided to EFSA No. 2). The reported limit of quantification (LOQ) for the analysis of the same element varied among the different producers.

Lead, arsenic and mercury in the analysed fumed SAS samples were below the LOQs (they ranged 0.005–2 mg/kg for lead, 0.1–0.6 mg/kg for mercury and 0.01–2 mg/kg for arsenic). The range of the LOQ values for analysed precipitated SAS samples were 0.03–1 mg/kg for lead, 0.02–0.6 mg/kg for mercury and 0.01–0.3 mg/kg for arsenic. The measured levels reported in precipitated SAS samples were for lead up to 3.1 mg/kg, 0.2 mg/kg for mercury and 0.2 mg/kg for arsenic. The ranges of the LOQs for SAS gel samples were 0.01–0.3 mg/kg for lead, 0.03–0.15 mg/kg for mercury and 0.004–0.75 mg/kg for arsenic. Measured levels reported in silica gel samples were for lead up to 0.62 mg/kg, 0.02 mg/kg for arsenic and below the LOQs for mercury. Data for which the reported LOQ was equal to the specification limit were not further considered. No data on hydrous SAS samples were reported.

Further analytical data on the presence of toxic elements in 17 samples (5 fumed, 11 precipitated and 1 silica gel) of E 551 by ICP-MS using an extraction method as described in JECFA for impurities soluble in 0.5 M HCl (2017) were submitted. This method uses a closed digestion system using 0.5 M HCl for 30 min at boiling temperature, followed by filtration through a 0.1-µm membrane filter (Documentation provided to EFSA No. 7). The LOQ values were 0.1 mg/kg for lead and arsenic, and 0.05 mg/kg for mercury. Levels of arsenic and mercury were below the LOQ in the 17 analysed samples (fumed, precipitated and SAS gel). In one of the 11 precipitated samples, lead was below the LOQ, while in the other samples the level for lead ranged from 0.1 to 2.1 mg/kg. The level of lead was below LOQ in the fumed and silica gel samples. No data on hydrous silica samples were reported.

A summary of the submitted analytical data on lead, mercury and arsenic using different methodologies is presented in Table 2.

TABLE 2 Summary of analytical data submitted for lead, mercury, arsenic.

		0.5 M HCl extraction method				Partial/full digestion		
		Pb	Hg	As		Pb	Hg	As
Type of SAS	**No**	**mg/kg**			**No**	**mg/kg**		
Fumed SAS	5	< LOQ[a]	< LOQ[b]	< LOQ[a]	7	< LOQ[c]	< LOQ[d]	< LOQ[e]
Precipitated SAS	11	< LOQ[a]–2.1	< LOQ[b]	< LOQ[a]	33[f]	< LOQ[g]–3.1	< LOQ[h]–0.2	< LOQ[i]–0.2
SAS gel	1	< LOQ[a]	< LOQ[b]	< LOQ[a]	11[j]	< LOQ[k]–0.62	< LOQ[l]	< LOQ[m]–0.02

Abbreviation: No, number of products analysed for each type of SAS.

[a]0.1 mg/kg.

[b]0.05 mg/kg.

[c]0.005–2 mg/kg.

[d]0.1–0.6 mg/kg.

TABLE 2 (Continued)

[e]0.01–2 mg/kg.
[f]Usually, 5 samples for each product, but in a few cases, 1 sample.
[g]0.03–1 mg/kg.
[h]0.02–0.6 mg/kg.
[i]0.01–0.3 mg/kg.
[j]Five samples for each product, but for one product, 4 samples.
[k]0.01–0.3 mg/kg.
[l]0.03–0.15 mg/kg.
[m]0.004–0.75 mg/kg.

The IBO indicated that the raw materials for E 551 are silicon-rich materials and chemicals (e.g. water glass, chlorosilanes), that are in turn generated by chemical processing of natural materials including silica sand. The impurities in the end-product E 551 come from the raw materials. Therefore, the IBO suggested that the specification limits for toxic elements need to take into account the variations of those impurities in the raw materials (Documentation provided to EFSA No.2).

The IBO indicated that variations in raw material sources can lead to variations in purity. Considering that the current limits for toxic elements in the EU specifications accommodate these variations, the IBO proposed to keep the same limits (Documentation provided to EFSA No. 2).

Following an EFSA request, the IBO provided analytical data on aluminium for different types of SAS (precipitated, fumed, gel and hydrous) used as E 551 (Documentation provided to EFSA No 22). Two sample preparation methods were used: full digestion with HF or extraction with 0.5 M HCl for 30 min. Analysis was performed by ICP-MS (DIN EN ISO 17294-2:2017-01) with a reported LOQ of 1 mg/kg and LOD of 0.3 mg/kg for both preparation methods. Different commercial products were analysed for each type of SAS and results were reported for three samples in most of the products. A summary of the results is presented in Table 3.

TABLE 3 Summary of the aluminium results reported for samples of SAS.

Type of SAS	No of products	Aluminium range (min–max) (mg/kg)	
		0.5 M HCl extraction method	Full digestion with HF
Precipitated SAS	9[a]	42–420	360–1500
SAS gel	4[a]	55–200	120–340
Hydrous SAS	1[a]	72–92	90–110
Fumed SAS	9[b]	< 1–2[c]	< 1[d]–8

Abbreviation: NA, not applicable.
[a]3 samples for each product.
[b]Only data in 3 products using full digestion with HF as sample preparation method.
[c]3 samples analysed only for 2 of 9 products, for the other products, 1–2 samples analysed for each.
[d]Internal company information based on analysis of 31 samples.

The Panel noted that the content of aluminium is very low in samples of fumed SAS (Table 3). The IBO explained that fumed SAS is produced from volatile chlorosilanes, which do not contain aluminium. On the other hand, in the wet process type, SASs are manufactured from a mixture of water glass solution and diluted acid. Water glass is made by melting sand, which is a source of aluminium, with soda ash, and for this reason, the content of aluminium in this kind of SAS is higher than in fumed SAS. Variations in sands of different origins used in the manufacturing are the main reason for the differences in aluminium content in the different products (Documentation provided to EFSA No. 22).

The IBO also indicated that HF completely dissolves the SAS, whereas the HCl extraction releases only leachable aluminium out of SAS, resulting in the observed difference between the reported content of aluminium depending on the sample preparation method used (see Table 2). While full digestion with HF more precisely accounts the total content of aluminium in SAS, the HCl extraction better mimics the conditions in the human digestive tract. Therefore, the IBO concluded that the HCl extraction, instead of the full digestion, should be the methodology to be used when determining toxic elements (Documentation provided to EFSA No. 22).

The IBO also indicated that HF completely dissolves SAS (releasing the full concentration of aluminium), whereas HCl extraction releases only leachable aluminium, resulting in the observed difference between the reported content of aluminium depending on the sample preparation method used (see Table 2). While full digestion with HF more precisely accounts the total content of aluminium in SAS, the HCl extraction better mimics the conditions in the human digestive tract. Therefore, the IBO concluded that HCl extraction, instead of full digestion, should be the methodology to be used when determining toxic elements (Documentation provided to EFSA No. 22).

3.2.3 | Particle size

According to the IBO (Documentation provided to EFSA No. 5), SAS types – fumed silica, precipitated silica and silica gel – are nanostructured materials with three levels of structure, as described below:

(i) Internal structures: The internal structures inside an aggregate originate from intermediately formed primary particles,[12] which are fused together during the manufacturing process. This fusion process is irreversible, and forms aggregates that cannot be redispersed to the stage of isolated primary particles. This is underlined by the fact that there are no phase boundaries inside an aggregate. Therefore, for synthetic amorphous silica materials, internal structures are a theoretical concept and do not satisfy the definition of a particle. The internal structures may be visually recognised by transmission electron microscopy (TEM) due to the curvature of the aggregate skeleton, but they cannot be isolated and unambiguously identified. In other publications (Albers et al., 2015 as indicated in Documentation provided to EFSA No. 5 and 9), these internal structures are also sometimes referred to as 'constituent particles', 'primary particles' or 'nodules';
(ii) Aggregates: The aggregates are the discrete entities with well-defined physical boundaries; hence, the aggregates represent the smallest dispersible units in synthetic amorphous silica;
(iii) Agglomerates: SAS products are placed on the market as micron-sized agglomerates. Depending on the densification and packaging process, these agglomerates can have diameters in the micrometre to millimetre range.

The Panel noted that the IBO used nomenclatures other than those recommended by Rauscher et al. (2019, 2023) and in line with the EFSA Guidance Particle-TR (SC Guidance, 2021a) to refer to the structure of the analysed materials. For harmonisation, the terms and concepts from Rauscher et al. (2023) are followed in this document.

Electron microscope (EM) pictures of the different SASs were provided (Documentation provided to EFSA No. 35) and presented in Figure 2.

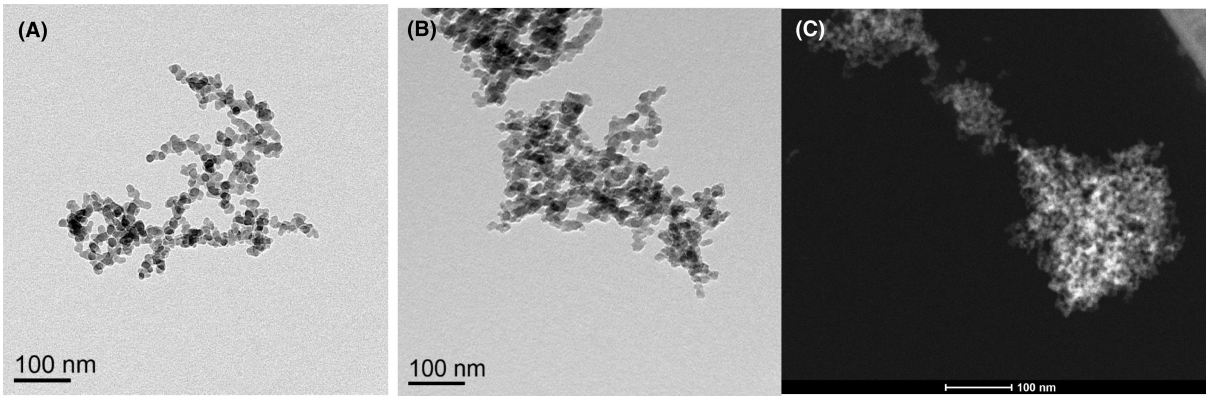

FIGURE 2 TEM image of (A) fumed SAS, (B) precipitated SAS and STEM image (C) SAS gel (Documentation provided to EFSA No. 35).

From the 70 different commercialised SAS (used as E 551), information on the size of the constituent particles analysed by TEM was provided only for 29[13] showing a range from 1 to 82 nm (Documentation provided to EFSA No. 2). The typical calculated Dmean and D50 sizes of the constituent particles range from ~2 to 30 nm and 2 to 28 nm, respectively (Table 4).

TABLE 4 Summary of the reported data[a] on the size of the constituent particles of SAS used as E 551 analysed by TEM.

	Number of products	Dmean[b] for constituent particles (nm)	D50[b] for constituent particles (nm)	Constituent particle size range (nm)
Fumed SAS	**4**	9–15	9–10	3–58
Precipitated SAS	**22**	2–30	2–28	1–82
SAS gel	**3**	6–15	6–10	2–40

[a]Values were rounded from the original data submitted (Documentation provided to EFSA No. 2.).
[b]Min–max values.

No data on constituent particle size analysed by EM were submitted for hydrous silica samples.

[12]The Panel considered that the terminology of primary particles used by the IBO refers to constituent particles in line with the terminology recommended by JRC (Rauscher et al., 2023).
[13]The Panel noted that no information on the constituent particle size analysed by TEM were submitted for products of one IBO.

According to the IBO, the manufacturing conditions for hydrous silica are the same as for SAS gel and only the water content is different. Therefore, the Panel considered that the constituent particle sizes in hydrous SAS are similar to those in SAS gel.

The IBO also provided information on particle size distributions of different SAS used as E 551 using laser diffraction (LD) (Documentation provided to EFSA No. 2 and 23). The Panel noted that the LD method is not considered a suitable method to investigate the presence of nanosized particles, as it does not provide information on the size of the constituent particles as required by the Guidance on Particle-TR and is prone to bias for polydisperse materials (Mech et al., 2020, Mech et al., 2020; Rauscher et al., 2019).

Analytical study quantifying the volume fractions in E 551

An additional report on an analytical study aiming at the quantification of volume fractions of sub-micrometre particles of SAS in aqueous suspension dispersed with ultrasonic probes for toxicology studies was submitted (Documentation provided to EFSA No. 7). These studies were performed with three commercial powders, each representing a principal type of SAS, namely fumed SAS, precipitated SAS and SAS gel. No hydrous SAS was tested. The results of three methodologies that measure the volume-based PSD covering a specific size range were combined to estimate the entire volume-based particle size distribution of the three types of SAS materials dispersed in ultrapure water (i.e. deionised and filtered through a 0.2-µm membrane). Different dispersion conditions (e.g. energy levels) were applied in different laboratories. An aqueous suspension containing SAS 133.3 g/L was diluted with ultrapure water to obtain a stock suspension with 1 wt.% SAS.

The dynamic light scattering (DLS) method was applied to measure the hydrodynamic diameter of particles from 0.05 to 1 µm. Angular light scattering (ALS) was applied to measure particle sizes from 0.5 to 200 µm. Gravimetric retention analysis using membranes with pore sizes in the order of magnitude of 1 µm was applied for size fractionation by membrane filtration, allowing a more accurate estimation of the PSD in the useful range of each method.

The optimal measurement concentrations depend on the optical configuration of the instruments, the design of the measurement cell and on the SAS product. For this reason, fumed SAS, precipitated SAS and SAS gel were analysed by ALS at optimal concentrations in the order of 0.1 wt.% and 0.01 wt.%, respectively. For DLS analyses, the stock suspension was initially diluted with ultrapure water at a mixing ratio of 1:99 (w/w), resulting in a dilute sample containing 0.01 wt.% SAS. A part of this sample was passed through a syringe filter containing a mixed cellulose ester (MCE) membrane with a nominal pore size of 0.8 µm. A procedure for combining the measurement results to construct the complete PSD from these results is provided and implemented, and measurement uncertainties of the different steps were estimated using a bottom-up approach.

According to the IBO, the method yields a volume-weighted size distribution for the size range of 10 nm up to approximately 100 µm. This was obtained by reconstructing the particle size distribution from all measurement results and has allowed deriving the target figures (i.e. volume fractions at 100 nm, 250 nm and 500 nm).

Proposal on specifications by the IBO

Based on the SOP 'Protocols for the granulometric characterisation of SAS particles in food-like suspensions' (Documentation provided to EFSA No. 3), applying the same methodology as presented in section above (Documentation provided to EFSA No. 7), the IBO proposed a description of the volume fraction of particles in E 551 measured by DLS as the basis for the specifications (Table 5).

TABLE 5 Volume fraction of particles in E 551 measured by DLS as proposed by the IBO (Documentation provided to EFSA No. 2).

Size	Volume fraction measured by DLS[a]
< 100 nm	< 10%
< 250 nm	< 90%
< 500 nm	< 100%

[a]Dispersion energy levels of 20–75 J/mL.

3.2.3.1 | *Particle size of E 551 in powdered food*

According to the IBO, E 551 is used as a free flowing and anticaking agent in powdered foods at low percentage addition levels. One of the technical functions of E 551 is to act as a spacer between food components, for them to remain in a free-flowing state (Documentation provided to EFSA No. 6).

Results of the analysis of SAS in a spice mixture, two salt mixtures, two tomato powder samples, a drink powder, a flavour powder and a buttermilk flavour powder were submitted (Documentation provided to EFSA No. 6). This includes scanning electron microscopy (SEM) pictures to show the distribution of SAS on the surface of the food, combined with the

corresponding energy-dispersive X-ray spectroscopy (EDX) spectral images that were taken to identify the SAS particles. TEM images of particles after dispersion were also provided.

The IBO concluded that the SEM data show SAS on the surface of the food matrices in the typical form of complex aggregates and agglomerates without any further breakdown. EDX analysis confirmed the presence of silicon on the surface of the food powder in aggregated structures. The presented examples illustrate that silicon is well distributed over the surface of the food.

The Panel noted that the applied approach and SEM equipment have technical limitations that limit drawing certain general conclusions drawn by the IBO:

- According to the IBO, a combination of 'SEM analysis and EDX-mapping at the macro-scale (note the scale bar '50 micrometre') in the silicon distribution' is applied with a lateral resolution of the applied configuration that is limited to the micrometre scale. Hence, the Panel noted that no conclusions can be drawn based on these results regarding the presence of small particles, including nanoparticles, and regarding possible breakdown of aggregates into particles in the nanoscale because the applied equipment cannot reach the required resolution;
- The IBO considered that SEM technology coupled with EDX would be the preferred method to describe SAS particle structure on the food matrix. The Panel noted that to demonstrate the absence of SAS nanoparticles, a better size resolution, considering the Merkus criterion (Merkus, 2009), is required. High-resolution SEM analyses or approaches based on TEM or scanning transmission electron microscopy (STEM) combined EDX would be more suitable;
- The IBO stated that using their approach 'a differentiation between added SAS and naturally occurring silica is not feasible, the EDX mapping is only identifying silicon.' The Panel noted that using more sensitive detectors, taking spectral images from high-resolution EDX analyses and implementing much smaller, particle-sized measurement boxes can overcome specific technical problems encountered (low resolutions, background and detector signals), and might strengthen or refute the statement of the IBO;
- The conclusions from the IBO that SEM data show SAS on the surface of the food matrices do not exclude the possibility of the presence of SAS inside the food matrices.

The Panel noted that, based on the information submitted to EFSA by an IBO or information from peer reviewed journals, no data on the relative amounts of particles occurring as isolated particles, aggregates and/or as agglomerates in food is available. In addition, no quantitative information on the level of aggregation/agglomeration of E 551 in food is available. Analysis of SAS in food is typically done after extraction from the food, which includes a sonication-based dispersion step aiming to deagglomerate the material.

Information on the aggregate size distribution and qualitative information on morphology of the aggregates for 16 SAS used as E 551 was available (Documentation provided to EFSA No. 35). Descriptive statistics (number based) were provided for the aggregate particle size based on Feret min measurement using quantitative TEM analysis for four fumed SAS and six precipitated SAS. The median sizes of the aggregates of fumed SAS were reported to range from 95 to 145 nm and for precipitated SAS from 42 to 212 nm. D10 and D90 based on Feret min ranged from 22 to 69 nm and 108 to 654 nm, respectively, among the 10 different SAS. The Panel noted that it is highly unlikely that only aggregates but also agglomerates were measured because in some pictures an overlap between aggregates can be observed. The Panel also noted that only a single measurement without uncertainty was reported for each SAS. Additionally, six SAS gels and one precipitated SAS were analysed in dispersed form by SEM/STEM; one of the SAS was also analysed as a dry powder. The Panel noted a high level of agglomeration on the provided images, which complicates identification and measurement of individual aggregates. This can result in a high uncertainty on the descriptive statistics related to the aggregate particle size that indicate a median size (number based) of the aggregates in the micrometre size.

3.2.4 | Information on the use of E 551 in different food applications

According to information submitted (Documentation provided to EFSA No. 27), the typical technical use of E 551 in the food industry is as an anti-caking agent and the typical processes used are mixing and spray-drying. As examples, mixing beverage powders with E 551 generally uses tumble blenders. E551 can be sprayed directly on a food during the spray-drying process where it sticks to the surface of the in situ formed granular particles of the food material. E 551 can also be mixed with a carrier oil and the oil dispersion then sprayed onto snack-food substrates. It was stated that E 551 used in food processing is subject to only low to moderate energy exposure during these typical processes like mixing and spray-drying.

According to further submitted information (Documentation provided to EFSA No. 23), a direct comparison of mixing energies in the food industry with energies coming from ultrasonic dispersion in liquids is not possible. Since agglomerated/aggregated E 551 particles larger than nanoaggregates are usually required to give the desired anticaking effects, the energy input during these mixing processes is much smaller than the energy used during sonication of aqueous dispersions (as indicated by Documentation provided to EFSA No. 27 based on Paul et al., 2004). These mixing processes are intended to keep the agglomerate/aggregate structure of E 551 intact, because grinding E 551 to smaller aggregates will diminish the efficiency for this application. When E 551 is used for carrier applications, a gentle mixing is required and optimum results cannot be obtained with high pressures or high shear forces, because they partly destroy the porous structure

and the absorptive capacity of E 551. The IBO indicated that usually slow and gentle mixing processes are used with mixing intensities far below ultrasonication in liquid media.

The Panel noted that a quantitative estimation of any dispersion brought about by the energy applied during the various food processing procedures is not feasible. It is expected that the level of dispersion in food is lower than the recommended protocols for dispersion of the test item for preparation of suspensions for toxicological studies (e.g. EU-funded Projects ENPRA, Nanogenotox, NANoREG) to obtain the most dispersed state as recommended in the EFSA Guidance on Nano – Risk Assessment (EFSA Scientific Committee Guidance, 2021b). A dispersion protocol applied in toxicological studies should ensure that the level of dispersion in the test system (in vitro or in vivo) is at least comparable to what can be expected in the food (EFSA Scientific Committee Guidance, 2021b).

3.2.5 | Technical data required for the risk assessment of silicon dioxide (E 551) for uses in foods for infants below 16 weeks of age

For the assessment of silicon dioxide (E 551) in infant formula (FC 13.1.1), as well as in special formulae used for infants under special medical conditions (FC 13.1.5.1) in infants below 16 weeks of age, the following was requested in the EFSA call for data2:

- information on the fate and the reaction products in the infant formula as ready to use;
- proposals for particular specification requirements for identity and purity in these food categories.

No information on the fate and the reaction products in infant formula as ready to use has been submitted to EFSA. The Panel noted that at the time of the re-evaluation (EFSA ANS Panel, 2018), IBOs indicated that E 551 is a rather inert substance and that no degradation products under normal conditions are known.

No proposal for particular specification requirements for identity and purity of E 551 in these food categories was provided. The Panel considered that information submitted for E 551 (Sections 3.2.1, 3.2.2 and 3.2.3) is considered also applicable to E 551 when used in infant formulae (FC 13.1.1) and in special formulae used for infants under special medical conditions (FC 13.1.5.1) in infants below 16 weeks of age.

3.3 | Dietary exposure to silicon dioxide (E 551)

3.3.1 | General population

Dietary exposure to silicon dioxide (E 551) for the general population was estimated at the time of the re-evaluation by the EFSA ANS Panel (2018). Considering that no new occurrence data are available to EFSA since 2018, and that dietary surveys from the EFSA comprehensive database giving the highest exposure estimates in the 2018 re-evaluation opinion are still in the consumption database, no new exposure estimation was performed for the general population.

The Panel considered appropriate to refer to the dietary exposure assessment performed by the ANS Panel from the 2018 opinion (see Table 6 in EFSA ANS Panel, 2018), and reconfirmed the choice of the refined, non-brand loyal scenario as the most appropriate and realistic scenario for risk characterisation of E 551 for the general population. 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.

It is noted that food belonging to FC 13.1.5.1 could be consumed also by infants above 16 weeks of age and young children (or toddlers). At the time of the re-evaluation of E 551, the ANS Panel did not perform an exposure assessment for the food for special medical purpose (FSMP) consumer only scenario (consumers of food under FC 13.1.5.1) since 'only one-use level was reported by industry on a niche product of FSMP described as special infant formulae'. Considering the current reported maximum level of 11.6 mg/kg for FC 13.1.5.1 (Documentation provided to EFSA N. 28), the Panel noted that the dietary intake for the infants above 16 weeks of age and young children (or toddlers) consuming FSMPs (FC 13.1.5.1) is in the same range of the dietary exposure estimated for infants and toddlers, brand-loyal scenario, at the time of the re-evaluation (EFSA ANS Panel, 2018) (further details in Annex E).

3.3.2 | Infants below 16 weeks of age

For the present opinion, the Panel performed a risk assessment for the age group of infants below 16 weeks of age, which was not performed at the time of the re-evaluation by the ANS panel.

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

The labelling of food additives authorised according to Annex III to Regulation No 1333/2008 is not mandatory; however, the online database Mintel's GNPD[14] was used for checking the labelling of silicon dioxide (E 551) on infant formula within the EU's food market. No baby formulae (0–6 months) were found to be labelled with silicon dioxide (E 551) in the last 5 years.

Information on the concentrations of E 551 in the ready-to-use infant formula was requested in the call for data[above2] for the re-evaluation of silicon dioxide (E 551) in infant formulae (FC 13.1.1), as well as, in special infant formulae for medical purposes (FC 13.1.5.1) for infants below 16 weeks of age.

Data were received from only one IBO on the use of E 551 as carry over in FC 13.1.1 and FC 13.1.5.1 formulae (Documentation provided to EFSA No. 28).

Dietary exposure to E 551 from its use as a food additive in formulae for infants below 16 weeks was estimated. This scenario is based on the recommended consumption levels from the applicable SC Guidance (EFSA Scientific Committee, 2017). This guidance 'recommends values of 200 and 260 mL formula/kg bw per day as conservative mean and high-level consumption values to be used for performing the risk assessments of substances which do not accumulate in the body present in food intended for infants below 16 weeks of age'. These recommended consumption levels correspond to 14- to 27-day-old infants' consumption.

The maximum permitted level of E 551 in the final infant formulae is not known; therefore, a regulatory maximum level exposure assessment scenario could not be estimated. The maximum levels provided by an IBO from both infant formulae and special infant formulae for special medical purpose, as well as the mean of all levels reported for each food category, were used for estimating the exposure to silicon dioxide (E 551).

Table 6 summarises the estimated exposure to E 551 from its use as a food additive in FC 13.1.1 and FC 13.1.5.1 for infants below 16 weeks of age.

TABLE 6 Dietary exposure to E 551 in infant formulae (FC 13.1.1 or FC 13.1.5.1) for infants below 16 weeks of age according to Annex III to Regulation (EC) No 1333/2008 (in mg/kg bw per day).

	Infants (< 16 weeks of age)
Estimated exposure assessment scenario	
Scenario using maximum level reported by industry (for FC 13.1.1 or FC 13.1.5.1, max level = 11.6 mg/kg)	
• Mean consumption (200 mL/kg bw per day)	2
• High-level consumption (95th percentile, 260 mL/kg bw per day)	3
Scenario using mean of levels reported by industry	
FC 13.1.1 = 4.1 mg/kg	
• Mean consumption (200 mL/kg bw per day)	0.8
• High-level consumption (95th percentile, 260 mL/kg bw per day)	1.1
FC 13.1.5.1 = 3.2 mg/kg	
• Mean consumption (200 mL/kg bw per day)	0.6
• High-level consumption (95th percentile, 260 mL/kg bw per day)	0.8

Abbreviation: bw, body weight.

Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainty have been considered and summarised in Table 7.

TABLE 7 Qualitative evaluation of influence of uncertainties on the dietary exposure estimates in infants below 16 weeks of age.[1]

Sources of uncertainties	Direction[a]
Consumption data for infants below 16 weeks of age: one reference point only to estimate exposure during the period of up to 16 weeks of age	+/–
Exposure assessment scenario for infants below 16 weeks of age: – Exposure calculations based on the reported levels (max and mean) as provided by industry	+/–

[a]+, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

E 551 is authorised at the level of 10,000 mg/kg in nutrient preparations in food for infants and young children (FC 13.1) according to Annex III to Regulation No 1333/2008.

There is uncertainty around the formula consumption values used since the actual formula consumption could be higher or lower than the standard values used in the scenario(s). There is also uncertainty on the reported levels by industry

[14]Mintel GNPD monitors new introductions of packaged goods in the market worldwide. It contains information of over 4.3 million food and beverage products of which more than 1,600,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 24 out of its 27 member countries, and Norway presented in the Mintel GNPD.

and used in the calculations. This gives rise to the potential both for possible over- and under-estimation of exposure (+/– in Table 7).

3.4 | Proposed revision to existing EU specifications

3.4.1 | Impurities

The potential exposure to impurities from the use of silicon dioxide (E 551) can be calculated by assuming that the impurity is present in the food additive up to a limit value, and subsequently calculation pro-rata to the estimates of exposure to the food additive itself.

The IBO provided analytical data on the levels of toxic elements and the Panel performed the risk assessment that would result if the toxic elements were present in E 551 at: (i) the existing maximum limit in EU specifications; (ii) at the levels from the analytical data reported with the HCl extraction method. For this second scenario, the rounded up highest measured value of the level of the toxic element was used, if quantified (this was only possible for lead 2.5 mg/kg and aluminium 450 mg/kg). In the absence of any measured value(s), the reported LOQ was used, after applying a factor of 5 to allow flexibility with respect to representativeness and homogeneity (i.e. resulting in 0.5 mg/kg for arsenic and 0.25 mg/kg for mercury). Further details of the outcome of the risk assessment for these scenarios is presented in Appendix E.

The Panel noted that the choice of the modulation factor and of maximum limits for toxic elements in the EU specifications is in the remit of risk management.

The resulting figures in Table E.4 (Appendix E) show that for lead and mercury, the presence of these toxic elements in E 551 either at the current specifications limit values or at the levels selected by the Panel would not give rise to concern, as well as for aluminium, considering the rounded up highest measured value. For arsenic, taking into account the use of E 551 for the general population, the calculated MOE values considering the current limit in the EU specification were considered to be insufficient.

Taking into account the calculations performed by the Panel (Table E.4) and the fact that the food additive is not the only potential dietary source of toxic elements, the Panel recommended to lower the maximum limits for lead, mercury and arsenic and to introduce a maximum limit for aluminium.

3.4.2 | Particle size and morphology

A report on an analytical study with three samples of E 551 (fumed silica, precipitated silica and silica gel) aimed at the quantification of volume fractions of sub-micrometre particles of SAS in aqueous suspension dispersed with ultrasonic probes was submitted (see Section 3.2.3) (Documentation provided to EFSA No. 5 and 23). The analytical approach combines three different methodologies that are based on the measurement of different physical properties and that each cover a specific size range. This approach allows estimating the entire volume-based size distribution of the three tested types of SAS.

The Panel noted the following limitations of the applied analytical methods based on light scattering (LS):

- The particles that are measured are aggregates/agglomerates. Constituent particles cannot be measured;
- Given the large polydispersity in size of the measured aggregated/agglomerated particles, biased measurements towards the larger particles can be expected within the application range of each method, although the size fractionation by microfiltration would be expected to reduce the bias somewhat;
- Volume-based size measurements assume a spherical morphology of the particles, whereas the presented SEM analysis shows a complex, fractal-like morphology of aggregated/agglomerated constituent particles;
- This methodology does not provide number-based size distributions as required in the EFSA Guidance (EFSA Scientific Committee, 2021a), and the results cannot be directly related to the number-based size distribution of the constituent particles.

Based on these limitations, the Panel concluded that the analytical approach and the volume-based size fractions to describe E 551 (Table 5) are not suitable in the context of implementing the EFSA Scientific Committee Guidance (2021a, 2021b).

The IBO has provided information on the constituent particle sizes measured by TEM for fumed SAS, precipitated SAS and SAS gel from different producers (see Section 3.2.3) that is summarised in Table 4. No information of the constituent particle sizes of hydrous SAS measured by TEM was submitted.

Based on the results of the data submitted, the Panel noted that the constituent particles in SAS used as E 551 are in the nanosize range with the majority exhibiting D50 from 2 to 28 nm (see Table 4).

The Panel considered that SAS used as E 551 consists of near-spherical constituent particles forming complex (fractal-like) aggregates as observed in the EM pictures submitted for samples of fumed SAS, precipitated SAS and SAS gel. No EM pictures on hydrous SAS were submitted. Taking into consideration the manufacturing process of hydrous SAS (see Section 3.2.1), the Panel expects the morphology of hydrous SAS to be similar to that of SAS gel.

Based on the information submitted by the IBO, the Panel considered that the specifications of the food additive should include the following:

- Fumed SAS, precipitated SAS, SAS gel and hydrous SAS are used as E 551.
- The chemical composition of SAS used as E 551 is SiO_2;
- SAS is an amorphous substance (without crystalline structure);
- SAS consists of near-spherical nano-sized constituent particles forming complex (fractal-like) aggregates, meaning that non-aggregated colloidal silica,[15] also referred as Stöber silica is not covered by E 551;
- SAS used as E 551 is not coated or surface functionalised.

Based on the data provided and information from the literature, it was noted that the constituent particle size varies more among the aggregates within a specific fumed SAS than between the aggregates of precipitated SAS. Moreover, it was also noted that the ramified tree-like morphology related to fractal dimension (Brasil et al., 2001) of aggregates between the fumed SAS can be different. According to information from the literature (e.g. Rubio et al., 2019; Spyrogianni et al., 2017), the degree of fusion between the constituent particles ('neck formation'), influencing properties like the specific surface area (SSA) of the material, depends on the synthesis temperature.

3.4.3 | Summary of the proposed revision to the EU specifications

Based on the information provided by the IBO and the above considerations, the Panel recommended the following revisions of the existing EU specifications for silicon dioxide (E 511). The Panel noted that the choice of amendment of the EU specifications is in the remit of risk management.

TABLE 8 Proposal for a revision of the existing EU Specifications for silicon dioxide E 551.

	Commission Regulation (EU) No 231/2012	Comments from the panel
NAME	Silicon dioxide	Synthetic amorphous silica*
Synonyms	Silica; silicium dioxide	SAS**
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	To be included that E 551 is not coated or surface functionalised and consists of near-spherical nano-sized constituent particles forming complex (fractal-like) aggregates
Assay	Content after ignition not less than 99.0% (fumed silica) or 94.0% (hydrated forms)	Content after ignition not less than 99.0% (fumed silica) or 94.0% (hydrated forms) of amorphous silica
Description	White, fluffy powder or granules. Hygroscopic	
Identification		
Test for silica	Positive	
Solubility	–	Insoluble/very slightly soluble in water
pH		
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)	
Loss on ignition	Not more than 2.5% after drying (1000°C, fumed silica) Not more than 8.5% after drying (1000°C, hydrated forms)	
Soluble ionisable salts	Not more than 5.0% (as Na_2SO_4)	
Arsenic	Not more than 3 mg/kg	Maximum limit to be lowered on the basis of the information provided by the IBO and on the considerations of the Panel To consider specifying HCl extraction as sample extraction method

[15]Colloidal silica preparations are 'stabilised colloidal suspensions' of silica nanoparticles in liquids, usually water and manufactured by different processes (ion exchange process with resins) (according to information provided during the re-evaluation of the E 551 (EFSA ANS Panel, 2018)).

TABLE 8 (Continued)

	Commission Regulation (EU) No 231/2012	Comments from the panel
Lead	Not more than 5 mg/kg	Maximum limit to be lowered on the basis of the information provided by the IBO and on the considerations of the Panel To consider specifying HCl extraction as sample extraction method
Mercury	Not more than 1 mg/kg	Maximum limit to be lowered on the basis of the information provided by the IBO and on the considerations of the Panel To consider specifying HCl extraction as sample extraction method
Aluminium		Introduce a maximum limit on the basis of the information provided and on the considerations of the Panel To consider specifying HCl extraction as sample extraction method

*The Panel considered that the current name 'silicon dioxide' is misleading since both crystalline and amorphous silicon dioxide forms, either natural or synthetic exist, and only synthetic amorphous silica is authorised as E 551.

**Based on the proposed change for the name, the current synonyms listed in the EU specifications are not appropriate and the only synonym/abbreviation for synthetic amorphous silica is SAS.

3.5 | Biological and toxicological data

The following was requested in the EFSA call for data2:

- In its 2018 opinion, the former ANS Panel highlighted several limitations to the available toxicological database of silicon dioxide (E 551) that would need to be decreased in order for EFSA to derive an acceptable daily intake (ADI) for silicon dioxide (E 551). There are a number of approaches which could decrease these limitations, which include, but were not limited to, a chronic toxicity study conducted according to a recognised guideline and with an adequately characterised material representative of silicon dioxide used as a food additive (E 551).
- Within the frame of the EFSA Guidance of the Scientific Committee on the risk assessment of substances present in food intended for infants below 16 weeks of age, the following information on the toxicological properties of silicon dioxide (E 551) is required:
  - Performance of an Extended One Generation Reproductive Toxicity Study (EOGRTS) in accordance with OECD TG 443 (OECD, 2018a).

The biological and toxicological data considered for this assessment consisted of a data set of 205 references comprising studies retrieved from (i) the published literature, (ii) data received from IBOs, following EFSA call for data and subsequent additional clarifications on the already submitted data, (iii) data spontaneously submitted by the IBO. The studies assessed at the time of the re-evaluation (EFSA ANS Panel, 2018) have been reconsidered in the current assessment, if published after year 2000 and relevant for the characteristic of the material, in the light of the progress in science and guidance for assessing the risk related to the exposure to nanomaterials.

The support of the EFSA cc WG genotoxicity was requested to review the evidence and conclude on the genotoxicity of E 551. A total of 59 genotoxicity studies have been assessed by the EFSA ccWG genotoxicity based on its approach for assessing reliability and relevance of genotoxicity studies (EFSA, 2023).

Regarding toxicity studies reporting endpoints other than genotoxicity, 146 references have been assessed independently by two members of the FAF WG follow-up Tox for relevance and internal validity. In vivo studies that were confirmed as relevant were individually appraised for their internal validity based on the OHAT Risk of Bias Rating Tool for Human and Animal Studies, which has been modified and used for the appraisal of both animal and human studies (see Appendix D).

Toxicity studies were considered for the assessment of E 551 if the test material was:

(i) the food additive silicon dioxide (E 551),
(ii) SAS material from the JRC repository (NM-200, NM-202, NM-203 and NM-204), which IBOs have indicated are representative of E 551 (Documentation provided to EFSA No. 7; Rasmussen et al., 2013),
(iii) SAS used in studies described in peer-reviewed publications for which the information in the publication regarding the physicochemical characterisation of the test item is limited, but indicates that the material consists of near-spherical nano-sized constituent particles forming complex (fractal-like) aggregates in line with the proposed definition of E 551 in the EU specifications (Section 3.4),
(iv) NM-201 or forms of SAS which are not used as E 551, but comply with the proposed definition of E 551 as indicated in (iii).

Toxicity studies performed with the materials under point (iii) and (iv) have been considered in order to identify whether specific information or restrictions should be included in the proposed amendment of the EU specifications for E 551.

The focus of the current assessment was to gather from the newly available evidence, any relevant information that could be used to refine the risk assessment and reduce the uncertainties identified by the ANS Panel in its earlier evaluation. In particular, the Panel examined whether new data could provide better estimates of kinetics and toxicity of E 551 after oral administration. The nanoscale considerations are taken into account in the section weighting the body of the evidence (Section 3.5.11).

3.5.1 | Absorption, distribution, metabolism and excretion

The toxicokinetics of E 551 have been investigated in vitro, using in vitro digestion studies and cellular systems, and in vivo studies. In the in vitro studies, the extent of the uptake and its mechanisms have been evaluated, in some cases using techniques that allow the visualisation of particles as small aggregates in the nano range. In some cases, fluorescence marked SAS, despite not being a relevant SAS used as E551, was used and the distribution of the fluorescence label was followed. In other studies, the Si concentration was measured as a surrogate for the concentration of SAS particles. In a small number of the in vivo studies, a few particles have been localised in organs. However, no quantification was possible and no data on the concentration–time profile of SAS particles was reported in blood or tissues. Si concentration measurements in blood and tissues were provided for different time points following single or repeated dosing in lieu of kinetic data of particles. The Panel acknowledged that Si concentration measurements in blood and tissues pose several challenges. The background Si concentration (i.e. the concentration in untreated animals) is high and variable due to the high Si content in the feed, which can vary between 50 and 5000 μg/g feed (Aureli et al., 2020). The LOQ of the methods is in the μg/g range (1–9 μg/g) depending on the tissue analysed, even with the more sensitive methods (Aureli et al., 2020). Therefore, the reported quantitative toxicokinetic results are surrounded by a not quantifiable uncertainty due to methodological issues. In some studies, the tested material was SAS not relevant for E551; nonetheless, the Panel considered relevant for the current assessment the information on the endocytotic uptake mechanism from these studies.

A detailed description of the in vivo studies is provided in Annex F.

In vitro dissolution and digestion

The IBO submitted several studies investigating the dissolution of different SAS in simulated gastric fluid (pH 3, 2 h, 36.4–37.7°C) and simulated intestinal fluid (pH 7, 2 h, 36.4–37.7°C) in a static test design (LAUS, 2020, 2023a, 2023b, 2023c; documentation provided to EFSA No. 21 and 24–26). The concentrations of dissolved Si were measured using inductively coupled plasma optical emission spectroscopy (ICP-OES). The Panel noted that the dissolution of the tested materials was low in simulated gastric and simulated intestinal fluid. The Panel further noted that more than 95% of the material was present in the small intestine as undissolved particles/aggregates.

Peters et al. (2012) analysed the amount of silica particles (5–200 nm) present in several food items (black coffee, instant soup, pancake) containing E 551 or with added SAS following a simulated digestion system; artificial saliva for 5 min (pH 6.5, 37°C), followed by treatment in artificial gastric juice (pH 2, 37°C) for 2 h; followed by 2 h in duodenal juice + bile (pH 8, 37°C). Samples at the different digestion stages were analysed by hydrodynamic chromatography coupled to inductively coupled plasma mass spectrometry (HDC-ICP-MS). The mass percentage of silica particles (5–200 nm), relative to the total amount of silica in the food item, was higher in coffee than in pancake or soup. For all food items, the percentage was higher at the duodenal juice stage (e.g. around 80% in coffee), followed by saliva and gastric juice (always < 5%). The authors also reported (by DLS and SEM) large silica agglomerates formed in the gastric digestion stage.

McCracken et al. (2013), incubated SAS (50 mg/L) with pepsin solution for 1 h (37°C) and after centrifugation re-suspended the pellets in pancreatin solution. After a 1-h digestion in the pancreatin solution (37°C) and centrifugation, the pellets were re-suspended in bile extract (1 h, 37°C) and finally in phosphate-buffered saline. The surface charge of the SAS changed in the pepsin solution and agglomerates were formed with radii of ∼3000 nm. Following treatment with pancreatin and bile salts, SAS deagglomerated and had radii of ∼109 nm with a zeta-potential in the range of −30 to −40 mV. The Panel considered this study not relevant because of the unphysiological conditions applied.

Lee et al. (2017) investigated the dissolution of E 551[16] in vitro in simulated gastric fluid and in vivo in the gastric fluid following oral administration of 500 mg (E 551) to rats. The Si concentration was measured using an unspecific method (molybdenum blue). In the in vitro study, 0.11 ± 0.04% of E 551 was dissolved and in vivo, in the gastric fluid of rats 0.19 ± 0.11% was dissolved. The dissolution of E 551 particles was not affected by the presence of albumin or glucose.

The aim of the study of Sohal et al. (2020) was to establish optimal conditions for performing in vitro studies in a model of the small intestinal epithelial barrier. In the context of this assessment, the results of E 551[17] dissolution in culture media are of interest. In cell culture media to which E 551 was added, after appropriate sonication, Si(IV) ion concentration

[16] As reported by the authors.

[17] Reported by the authors as Aerosil-200F which according to Appendix A of the EFSA ANS Panel (2018) is used as E551.

increased considerably, reaching 40.7% at 24 h and 42.8% at 48 h. The Panel noted that the findings may indicate a marked dissolution of E 551 in culture media.

In the publication of Zaiter et al. (2022), in vitro digestion experiments were performed with E 551.[18] The authors successively incubated E 551 in gastric fluid (pH 1.5) for 2 h and then in intestinal fluids (pH 6.8). They recovered the particles thereafter and compared the properties after digestion with the properties before ('native' E 551). No agglomeration of the 'digested' particles were observed and digestion did not modify zeta potential, isoelectric point and specific surface area. TEM analysis of both samples showed constituent particles with a mean size near 14 nm.

In vitro studies

Chu et al. (2011), studied endocytosis and exocytosis of fluorescence labelled Stöber silica particles of 50 nm (silica not used as E 551) in various cell lines. Endo- and exocytosis was observed by TEM and confocal microscopy when cells were incubated for 10–48 h. The authors describe non-specific endocytosis as a temperature-dependent mechanism for cellular uptake of the SAS small aggregates. Small aggregates are mainly found in intracellular, membrane bound organelles; only a few small aggregates were seen in the cytoplasm, indicating that obviously these organelles do not rupture. The small aggregates are excreted from the cells via exocytosis which does take place simultaneously with the endocytosis. The amount of small aggregates inside the cell is dependent on the amount of small aggregates outside cells in the medium. Endocytosis was not quantified. The Panel considered that the principle of endo- and exocytosis and the intracellular behaviour of these silica particles in the cells were well described although it remains unclear to which extent the observations with this kind of silica particles can be extrapolated to E 551 and the in vivo situation.

McCracken et al. (2013), studied the internalisation of commercially available SAS (constituent particle size 12 nm) using the intestinal epithelial cell line C2bBe1, a subclone of Caco-2 cells which forms polarised monolayers of cells with microvilli maintained by cytoskeletal proteins. Cells were treated with 10 μg/cm^2 of SAS. After 24 h, cells were processed for TEM. Clustered SAS were visualised inside a fraction of the C2bBe1 cells. Particles were observed only in the cell cytoplasm and not in the nucleus. The Panel noted that no confirmation on the identity of the particles, observed by confocal microscopy and TEM, was available in the publication.

Yang et al. (2014) investigated the cellular uptake of four E 551[19] with constituent particle sizes between 10 and 50 nm by TEM. Uptake in the cells was investigated in suspension cultures of GES-1 and of Caco-2 cells. Following incubation at a final concentration of 200 μg/mL for 24 h, the cell ultrastructure remained unchanged, except for swelling of the mitochondria, compared to controls without exposure to E 551. In both GES-1 and Caco-2 cells, particles in the nano range were found in endocytic vesicles in the cytoplasm. The uptake of the four different E 551 was comparable. The presence of particles in the nano range inside cells is also confirmed with EDX spectroscopy, showing peaks of O and Si. Translocation of particles in the nano range was investigated in a monolayer culture of Caco-2 cells. E 551 samples dispersed in HBSS at 200 μg/mL were added to the apical side; after 4 h of exposure, the silicon concentration was measured by ICP-OES in the media on the basolateral side. For all E 551, silicon was not detectable, indicating that the particles in the nano range present in E 551 did not pass through the Caco-2 monolayer after 4 h contact. The Panel noted that uptake of E 551 was demonstrated in suspension culture and an exposure time of 24 h. No uptake could be demonstrated for a Caco-2 monolayer culture and an exposure time of 4 h.

In the study of Halamoda-Kenzaoui et al. (2017), Caco-2 cells were used to examine the uptake and its mechanisms with fluorescence-labelled SAS, not used as E551, of 30 and 80 nm, at a concentration of 200 μg/mL applying flow cytometry, TEM, inhibitors of endocytosis and gene silencing. In this study, the materials were analysed either freshly dispersed or after 24 h incubation to allow the agglomeration. The uptake was not quantified in terms of concentration of Si or SAS particles but by relative fluorescence only. In the TEM figures, obtained after 6 h exposure, clusters of nanoparticles in endosomes and lysosomes, membrane ruffling which is typical for macropinocytosis and membrane invaginations typical for endocytosis were seen. The extent of cellular uptake and the mechanism of endocytosis of this silica nanoparticles were dependent on their agglomeration state, with increased nanoparticle agglomeration enhancing cellular uptake. Uptake was temperature-dependent, indicating energy dependence of the process. The Panel noted that this silica is taken up by cells and found in endosomes and lysosomes, after 6 h of exposure, through a temperature-dependent (energy-dependent) mechanism of macropinocytosis and endocytosis. It remains unclear to which extent the observations with this kind of silica particles can be extrapolated to E 551.

Zhang et al. (2017), used a Caco-2 model to study the transport of fumed E 551[20] and a silica gel not used as E 551 to the basolateral side which was 0.09% and 0.06% of the donor amount after 3 h and 0.25% and 0.13% of the donor amount after 6 h for fumed E 551 and silica gel, respectively. The silica uptake by the Caco-2 cell monolayer was visualised by confocal images focusing on the nucleus layer. After 6 h incubation, the Caco-2 cells contained more fumed E 551 particles than silica gel particles. The Panel noted that this study showed some differences in the cellular uptake of fumed E 551. While the observed cellular uptake differed only by a factor of 2, the data suggest that only a small amount of Si can cross the Caco-2 cells and reach the basolateral side.

[18] As reported by the authors.

[19] As reported by the authors.

[20] Reported by the authors as Aerosil® 200 which according to Appendix A of the EFSA ANS Panel (2018) is used as E551.

Lee et al. (2017) investigated the transport mechanism of E 551[21] and an SiO_2 (4 ± 1 μm) not used as E551 across the intestinal epithelium in an in vitro model of Caco-2 cells co-cultured with human Burkitt' lymphoma Raji B cells, which induce the specialised microfold (M) cells in Peyer's patches. The transport of particles from E551 and SiO_2 by M cells was temperature-dependent, and the transport of E 551 particles was higher than that of SiO_2. In the Caco-2 cell system without Raji B cells, no particles from E551 and SiO_2 were transported. EGTA treatment which induces the opening of enterocyte tight junctions had no influence on particles from E551 and SiO_2 transport. The Panel noted that M cells, specialised enterocytes present in the follicle-associated epithelium (FAE) of Peyer's patches, were taking up particles from E551 and SiO_2 dependent on temperature and that the transport is not through the intercellular space.

García-Rodríguez et al. (2018) used a modified three-dimensional in vitro intestinal epithelial model Caco-2/HT29/Raji-B to study the transfer of NM-203, constituent particle size 24.7 nm, dispersed according to the EU Nanogenotox protocol (ANSES, Nanogenotox, 2011) into the basolateral chamber. The co-cultures were exposed for 24 h to 50 μg/mL. Confocal microscopy analysis was performed to locate the small aggregates in the nano range. The data show that the nanoparticles (nanoaggregates) are mostly located in the mucus layer. Some nanoparticles are seen in the cytoplasm of the cells and in the cell nucleus. The Panel noted that no confirmation was provided that the particles, observed by confocal microscopy contained SiO_2.

In the study of Yu et al. (2020), intestinal transport of 13 different E 551[22] and a commercial SAS was investigated by different models. Raji B cells were added to basolateral inserts of Caco-2 cells to form a co-culture. The apical medium of co-culture monolayers was replaced by a medium containing particles (500 μg/mL) and incubated for 6 h. Quantitative analysis of transported silica was performed in the basolateral solutions. A Caco-2 monoculture model was used to evaluate the transport of particles through the intestinal epithelial tight junctions. An in vitro FAE model and Caco-2 monolayer, representing M cells in Peyer's patches and intestinal tight junctions, were used to evaluate the intestinal transport efficiency of silica particles and the mechanism. The concentration of silicon from SAS was determined by molybdenum blue spectrophotometric method. SEM and TEM images were used to characterise the particles. The results showed that silica was taken up by M cells irrespective of the particle size. No intestinal transport was observed of the food additives investigated. SAS transport was seen in both models, uptake by M cells and passing the Caco-2 monolayer, indicating different intestinal transport mechanisms for SAS and E 551. The Panel noted that M-cells in Peyer's patches take up silica particles of food additives. SAS was also taken up by passing the Caco-2 monolayer. The extent of the uptake was dependent on the size distribution and did not differ between the tested food additives.

Zaiter et al. (2022) incubated a co-culture of HT29-MTX and Caco-2 cell lines covered by a continuous mucus layer. The culture was incubated with fluorescence-labelled colloidal silica particles (20–200 nm), not used as E 551, for between 2 and 24 h. Most of the fluorescence label was found in the mucus layer of the cell culture; some label connected with the small particles with constituent sizes of 20 and 30 nm was found intracellularly. Incubation for 24 h increased the fluorescence in the cell for both nanoparticles of 20 and 30 nm. The Panel noted that the mucus layer can play an important role in hindering the particles to reach the intestinal cell surface and to be taken up into the cell. It remains unclear to which extent the observations with this kind of silica particles can be extrapolated to E 551.

Tanaka and Ogra (2024), used colloidal silica, not used as E 551, of five different sizes (10, 30, 50, 70, 100 nm) to study the uptake in human hepatoma HepG2 cells. Si measurements were performed by ICP-MS. The cells were exposed to the silica nanoparticles diluted in Milli-Q water to 2.0 μg/mL each. In addition, a mixture containing all the investigated silica nanoparticles in a concentration of 2.0 μg/mL was also prepared. The uptake was visualised by TEM after an exposure time of 1 h and exposure to 50 μg/mL of 10 nm and 100 nm silica nanoparticles. According to the Si concentration, more small than large nanoparticles were absorbed by the cells (10 nm: 46%; 30 nm: 34%; 50 nm: 23%; 70 nm: 15%; 100 nm: 11%). Using TEM, several large agglomerates ranging from 100 to 1000 nm and consisting of small nanoparticles were observed in the cells exposed to 10 nm silica nanoparticles. The results suggest that silica nanoparticles agglomerate to form secondary particles with sizes > 100 nm in the culture medium. TEM images of the cells exposed to 100 nm silica nanoparticles revealed also the presence of agglomerates inside the cells. Agglomerates were mainly found close to the cell surface in this study, possibly because of the short exposure period of 1 h. The Panel noted that particles with a smaller constituent size (10 nm) are taken up to a greater extent than particles with a higher constituent size (100 nm), although both were found in aggregate form. It remains unclear to which extent the observations with this kind of silica particles can be extrapolated to E 551.

Kwon et al. (2024) investigated the cellular uptake of fumed E 551[23] and precipitated E 551[24] in Raw 264.7 cells (a murine macrophage cell line). Uptake was measured by quantifying total cellular Si levels using ICP-OES after exposure for 24 h at a dose of 1000 μg/mL. The intracellular particles were separated via the cloud point extraction (CPE) approach from the soluble silicic acid and from dissolved ionic form. The soluble forms were determined using the molybdenum blue assay and ICP-OES analysis, respectively. At the measured time points (2, 6 and 24 h), the silica particles accounted for 83.9%–87.3% of the total Si concentration, the Si ion for 8.9%–12.9% and the silicic acid for 2.1%–5.7%. The uptake of precipitated E 551 was somewhat smaller than that of the fumed E 551. The content of total Si appeared to decrease with time despite continued exposure. At 24 h, the total Si concentration was only 41.4% and 51.8% of the concentration at 2 h for the fumed

[21]As reported by the authors.

[22]As reported by the authors.

[23]Reported by the authors as Aerosil® 200 which according to Appendix A of the EFSA ANS Panel (2018) is used as E551.

[24]Reported by the authors as Sipernat® 22 which according to Appendix A of the EFSA ANS Panel (2018) is used as E551.

and the precipitated E 551, respectively. The authors did not comment and did not explain the findings. The Panel also noted that the Si concentration as Si ion and of silicic acid in the medium has not been followed which might explain the intracellular loss of material. Intracellular Si was found in ionic form and as silicic acid suggesting that, after cellular uptake, E 551 particles can dissolve into Si ions but also transform to silicic acid. Because the findings using the CPE method could not be quantitatively confirmed, there was some uncertainty related to the quantitative data on the forms of Si in the cells.

In summary, the Panel noted that not all in vitro studies were performed with SAS used as E 551. Despite this fact, the Panel considered that from the findings some principles of uptake and their mechanisms can be obtained which are also valid for E 551. Based on the reported data, the Panel concluded that silica can be taken up by cells by temperature-dependent pinocytosis, indicating an energy-dependent uptake mechanism. The extent of the uptake was small and particle size-dependent, with the majority of publications reporting that smaller particles are taken up to a greater extent than larger particles. In cell culture systems, mirroring cells of the small intestine with mucus production, it was shown that the mucus may hinder the uptake of particles. One publication reported that the Si concentration in the cells is mainly due to intracellular particles and that a small portion is contributed by the Si ion and another portion from silicic acid. The Panel noted that the intracellular Si concentration declined with time to nearly 50% and that the concentration of Si ion and of silicic acid in the medium has not been followed which might explain the intracellular loss of material.

In vivo studies

Mice

In mice, three studies investigated tissue distribution and persistence of silica, not used as E 551, in the body after intravenous (i.v.) administration (Morishita et al., 2012; Yamashita et al., 2011; Yu et al., 2013). In Yamashita et al. (2011), after administration of silica (70 nm), nanoparticles were identified in placental and fetal tissue (liver, brain). In Morishita et al. (2012), after administration of silica (70 nm), nanoparticles were detected in the cytoplasm and nuclei of Sertoli cells, spermatids and spermatocytes. In the study of Yu et al. (2013), after administration of silica (64 nm), nanoparticles in the liver (Kupffer cells, hepatocytes), in the lung and the spleen (macrophages), in the kidney (capillary endothelial cells) and in the myocardial interstitium were observed. In all three studies, high concentrations of nanoparticles were found in the liver or spleen and low concentrations in the other tissues investigated, such as heart, kidney and brain. TEM was used for the analysis in all studies, however without verification by EDX that the observed particles correspond to silica. When silica of larger sizes (300 and 1000 nm) was administrated, particles were only identified by the presence of their fluorescence label in liver but not in other investigated tissues (Yamashita et al., 2011).

In the study by Yu et al. (2013), analysis of Si by ICP-OES showed that about 50% of the silica dose was found in the tissues on day 14, measured. From this information, the Panel deduced that the half-life of this kind of silica particles is 14 days. The accumulation factor,[25] calculated by the Panel, is 21 which means that the peak concentration and the area under the curve at steady state, which would be reached by repeated daily administration for 84 days, is 21 times higher than after a single administration of the same dose.

Four studies (Boudard et al., 2019; Lu et al., 2021; Yan et al., 2020; Zhan et al., 2024) investigated tissue distribution and persistence of SAS in the body after oral ingestion.

In Boudard et al. (2019) study, NM-200 was given for 18 months by drinking water. The exact dose is not reported but amounts to about 4.8 mg/kg bw per day according to the authors. The tissue content of Si was measured by inductively coupled plasma triple quadrupole mass spectrometry (ICP-QQQ-MS). The number of animals was limited (4–6 for the control and 4–7 for the treated group) and the variability in the results is high. No statistical difference was consistently seen between the Si concentration in liver and kidney of treated animals and controls.

In the study of Yan et al. (2020), animals were administered by gavage with SAS at 25, 160 and 300 mg/kg bw per day for 28 days. The content of Si was measured by ICP-OES in cerebral cortex, heart, liver, stomach, duodenum and colon tissue of the 160 mg/kg bw per day group and the control group. No statistical difference was seen between the Si concentration in the tissues of these animals dosed with 160 mg/kg bw per day group and the control animals with the exception of the colon, where the concentration in the silica-treated group was significantly higher than in the control. The data of this study show that, if SAS is absorbed at all, the absorption would be extremely low. No further information concerning the ADME can be obtained from this publication.

In the study of Lu et al. (2021), fumed E 551[26] and precipitated E 551[27] were studied in male BALB/c mice. An investigation on the behaviour of nanoparticles during in vitro digestion of feed matrices showed that after a decrease of nanoparticles in the gastric phase at pH 2 more than 96% of precipitated E 551 and fumed E 551 were present as silica particles in the intestinal phase. Si concentrations in organs were measured by ICP-OES following 28 days and 84 days continuous oral administration. After 28 days of treatment with 225, 1000 or 5000 mg/kg bw of precipitated E 551 and fumed E 551, no Si was accumulated in the tested organs. Following 84 days of administration, the Si concentration in the lung, kidney, stomach and intestine was not different from control levels. However, the Si concentration in the liver and spleen was higher than in the control for all doses and both types of E 551.

[25]Cumulation factor: $CF = 1 - e^{-kt}/1 - e^{-k\tau}$ where ke = elimination constant = ln 2/half-life, t = duration of the study, τ = dosing interval. For Si half-life = 15 days, ke = 0.0462/day.

[26]Reported by the authors as Aerosil® 200 which according to Appendix A of the EFSA ANS Panel (2018) is used as E551.

[27]As reported by the authors.

The Panel calculated the content of fumed E 551 and precipitated E 551 in the liver and spleen by multiplying the Si concentration in the organ with the organ weight (taken from public sources) and the sum of the content was compared to the cumulative intake, calculated by a median body weight taken from the publication. The accumulation could be estimated to decrease with the dose being 0.02%, 0.003% and 0.0008% and 0.008%, 0.0016% and 0.0005% for dose levels of 225 mg/kg bw, 1000 mg/kg bw and 5000 mg/kg bw of precipitated E 551 and fumed E 551, respectively, indicating a low accumulation of E 551.

Absorption was also calculated by dividing the sum of the organ Si contents after multiple continuous dosing by the cumulation factor. For precipitated E 551, this resulted in an absorption of 0.1811%, 0.0315% and 0.0068% of the dose at 225, 1000 and 5000 mg/kg bw per day, respectively, while for the same dose levels of fumed E 551 absorption values of 0.0663%, 0.0134% and 0.0041% of the dose were determined. These estimates indicated low systemic availability/absorption. Thus, for the external doses of 225, 1000 and 5000 mg/kg bw per day, internal doses of 0.407, 0.315 and 0.340 mg/kg bw per day for precipitated E 551, and 0.149, 0.134 and 0.205 mg/kg bw per day for fumed E 551 can be derived. The values showed that the internal dose does not increase proportionally to the external dose and that differences between different SAS used as E 551 may exist.

In the study of Zhan et al. (2024), pregnant BALB7c mice were treated with 225 mg/kg bw or 5000 mg/kg bw of fumed[28] E 551 and precipitated E551[29] from GD1 to GD19 (18 days). After delivery, the identity of silica-containing particles was confirmed by SEM–EDX as well as micro-X-ray fluorescence in maternal and fetal livers ($n = 4$); only data for 5000 mg/kg bw were shown. No particles were observed in the livers of the control group. The Si concentration was measured by ICP-OES in maternal and fetal livers ($n = 4$) and was found to be increased compared to controls only for the dose of 5000 mg/kg, but not for the dose of 225 mg/kg bw per day. This might be explained by the high background and the short duration of the dosing.

Rats

In rats, four studies investigated tissue distribution and persistence in the body (Zhuravskii et al., 2016; Waegeneers et al., 2018; Nanogenotox project study (De Jong et al., 2013) and Aureli et al., 2020; Tassinari et al., 2021) of SAS after i.v. administration. In the studies, high concentrations were found in liver and spleen and lower concentrations in other tissues investigated such as heart, kidney and brain.

In the study of Zhuravskii et al. (2016) with fumed SAS, the presence of particles in hepatocytes was demonstrated by TEM and the confirmation that the particles contained Si was performed with EDX. From the decline of the Si concentration in liver and spleen in the study of Zhuravskii et al., a half-life of 30 days and 15 days, respectively, can be estimated.

In the study of Waegeneers et al. (2018), with i.v. administration of NM-200 20 mg/kg, distribution and urinary excretion of Si were measured. Liver and spleen were the organs with the highest Si content (liver 64 ± 22%, spleen 1.5 ± 0.6% of the dose at 6 h). The urinary excretion of Si was 5 ± 2% of the silicon dose at 6 h and increased to 32 ± 7% at 24 h after injection whereas the Si content in the liver declined to 54 ± 14% of the dose, and that in the spleen to 1.0 ± 0.7%.

In the Nanogenotox project study (De Jong et al., 2013) and follow-up publication by Aureli et al. (2020), NM-200 or NM-203 were tested at a total dose of 100 mg/kg bw, divided into five consecutive i.v. injections of 20 mg/kg each. High concentrations of Si were measured in liver, spleen and lung by a specified ICP-MS employing dynamic reaction cell technology to overcome polyatomic interferences (dynamic reaction cell inductively coupled plasma mass spectrometry (DRC-ICP- MS)). The sum of the Si amount in liver and spleen was 0.7% of the dose at 90 days following injection of NM-203. From the decline in tissue content the Panel calculated a half-life of SAS of roughly 15 days, resulting in an accumulation factor[30] of 22. In the study of Tassinari et al. (2021), NM-203 was intravenously administrated in the tail vein as a single dose of 20 mg/kg bw or as a cumulative dose of 100 mg/kg bw (5 administrations 1 day apart). At day 90 following administration, the content of Si in the liver and spleen was measured. In the treated animals of the repeated dose group, the Si content in the liver was 11 mg/kg for males and 20 mg/kg for females, and in spleen, it was 28 mg/kg for males and 24 mg/kg for females.

Eleven studies investigated tissue distribution and persistence in the body of SAS after oral administration (Zhang et al., 2017; Tassinari et al., 2020; Yoo et al., 2021; Liang et al., 2018; Lee et al., 2017; Yoo et al., 2022; van der Zande et al., 2014; Nanogenotox project study (De Jong et al., 2013) and Aureli et al. (2020), Kwon et al. (2024), Fraunhofer (2011, Documentation provided to EFSA No. 14); AnaPath, 2020 (Documentation provided to EFSA No. 10).

Zhang et al. (2017) investigated the absorption of fumed E 551[31] and a silica not used as E 551 (particle size 3084 nm, 'micro silica') in vivo in tissue from different parts of the small intestine and in vitro in a CaCo-2 model. In the in vitro study, the Si concentrations were measured by ICP-OES. To observe the cellular uptake of the silica, confocal laser scanning microscopy was used. Both materials were given to male rats by intragastric administration at a dose of 250 mg/kg bw. The small intestine was obtained 6 h after administration when the administered material had passed through the small intestine. More E 551 particles than 'micro silica' particles adhered to the mucous membrane and were taken up by the cells. The particles did disperse in the cytoplasm but did not enter the nucleus. A large amount of E 551 was taken up by cells of the Peyer's Patches whereas nearly no 'micro silica' was seen in the ileum. E 551 was transported across the surface of the Peyer's Patch cells and reached a depth of 10–15 µm. The authors conclude that smaller particles (E 551) are better transported through the cell membranes in the gastrointestinal tract than larger particles ('micro silica').

[28]Reported by the authors as Aerosil® 200 which according to Appendix A of the EFSA ANS Panel (2018) is used as E551.

[29]As reported by the authors.

[30]Cumulation factor: $CF = 1\text{-}\ e^{-kt}/1\text{-}e^{-k\tau}$ where ke = elimination constant = ln 2/half-life, t = duration of the study, τ = dosing interval. For Si half-life = 15 days, ke = 0.0462/day.

[31]Reported by the authors as Aerosil® 200 which according to Appendix A of the EFSA ANS Panel (2018) is used as E551.

In the Tassinari et al. (2020), six treatment groups ($n = 8$/sex/group) were administered NM-203 at 2, 5, 10, 20 or 50 mg/kg bw per day by gavage on 5 days/week for 90 days. A control group was treated with vehicle only. Distribution in liver, spleen, small intestine and brain was assessed by determining the Si concentration using a sensitive and specific method (ICP-QQQ-MS). Statistically significant higher Si concentrations were detected in female rats for only two tissues, each at a single dose level (liver at 5 mg/kg bw and spleen at 50 mg/kg bw) without confirmation from the other dose groups. Differences in Si concentrations in the liver and spleen of male animals and in small intestine and brain of both sexes at all dose levels were not statistically significant when compared with the control. From this study, the Panel considered that NM-203 is only absorbed/systemically available to a not measurable extent when administered by the oral route in very low doses up to 50 mg/kg bw per day.

In the study of Yoo et al. (2021), fumed E 551[32] and of precipitated E 551[33] were given by gavage to female rats ($n = 4$ per group) in a single dose of 300 mg/kg bw (precipitated E 551) and 2000 mg/kg bw (fumed E 551). Blood, gastric fluid and tissue samples from kidney and liver were collected at the time of peak concentration (300 mg/kg, 2 h; 2000 mg/kg, 10 h). Si levels were analysed by ICP-OES. Less than 0.2% of the dose was found in the gastric fluid, with no significant differences between administered doses or type of E551. At these time points, the majority (~ 81%) of precipitated E 551 and fumed E 551 in the liver and blood was in the ionic forms. In the kidneys, all Si determined was in the ionic form.

In the study of Liang et al. (2018), precipitated SAS was administered in doses of 0, 166.7, 500 and 1500 mg/kg bw per day for 13 months by gavage (10 male and 10 female rats per group). At the end of the experiment, the content of Si was measured by ICP-OES (no information on LOD and LOQ was reported) in whole blood, liver, kidneys, and testis, collected from five rats of each sex per group. The variability in the results is large and no statistical difference was seen between the Si concentration in the tissues of the control animals and the treated animals. From this study, the Panel concluded that absorption/systemic availability of the tested material (precipitated SAS) by the oral administration occurs only to an extent that is not measurable with the applied method.

In the publication from Lee et al. (2017), E 551[34] was studied following oral administration of 500 mg/kg to rats. Si concentration was quantified by the molybdenum blue method in blood, kidney, liver, lung and spleen. The AUC was higher for E 551 with albumin or glucose solution than following administration of E 551 in distilled water. The authors reported half-lives between 2.82 and 3.40 h which seem to be distribution rather than excretion half-lives. The authors calculated the extent of absorption from the AUC without explaining the procedure. Because no data following intravenous administration are available in this publication for comparison, the Panel considered these values for the absorption to be incorrect. For the tissue distribution and excretion kinetic study, 500 mg/kg of E 551 in 1% glucose was orally administered. Compared to untreated controls, total Si levels were elevated in kidneys, liver, lungs and spleen of the rats administered with E 551. Elevated Si levels in lungs returned to control levels on the second day after administration, in other organs, it took 3–4 days. Elevated Si levels in urine and faeces returned to control levels after 4–5 days. The authors stated that 5.02 ± 0.32% of E 551 was excreted via urine while excretion via faeces was 84.54 ± 8.57%, however without considering the Si-excretion in the control group. When correcting the excretion values by the values from the control group, the urinary excretion in the treated group is ~4 mg, equal to 11.4% of the Si-dose. These data are in contrast to the absorption of between 1.54% and 3.94% that the authors claim from the AUCs. Excretion following Si dosing is reported to be ~190 mg whereas the Si-dose is 35 mg.[35] Thus, the sum of Si excretion by faeces and urine exceeds the Si-dose. The Panel considered the data from this study to be implausible.

In the Yoo et al. (2022), fumed E 551[36] and precipitated E 551[37] were studied in a single dose toxicokinetic study and in a 28-day repeated dose distribution and organ concentration study in female Sprague–Dawley (SD) rats at oral doses of 50, 300 and 2000 mg/kg bw ($n = 6$ per group for the single dose study and $n = 5$ for the repeated dose study). In this toxicokinetic study, plasma Si concentrations were determined after microwave digestion by ICP-OES analysis. No information on the LOD and LOQ was reported. Higher plasma Si concentrations were measured with increasing dose. C_{max}, T_{max}, half-life, MRT, Cl/f, AUC were calculated by a modelling programme. The authors calculated the absorption using the dose and the AUC values without indicating which calculation they performed. The Panel considered that the reported half-lives and MRTs for the doses of 50 and 300 mg/kg are distribution half-lives rather than elimination half-lives and are influenced by the fact that Si concentrations could not be measured anymore after 4 and 6 h for 50 and 300 mg/kg, respectively. The Panel considered that the calculation of the absorption is not correct because the authors do not have data from an intravenous study arm for comparison.

Si concentration measured in the liver, kidney, mesenteric lymph node, stomach and small intestine after 28 days of treatment with 50 and 300 mg/kg bw was no different from control. After 28 days of administration of a dose of 2000 mg/kg bw per day, there were no difference compared with controls in the kidney, mesenteric lymph node, stomach and small intestine. The liver Si concentration was higher than in the control for fumed E 551 and precipitated E 551. However, the authors report that on the first day of the recovery period (day 29) no difference to the control value in the liver was observed, which

[32]Reported by the authors as Aerosil® 200 which according to Appendix A of the EFSA ANS Panel (2018) is used as E551.

[33]Reported by the authors as Sipernat® 22 which according to Appendix A of the EFSA ANS Panel (2018) is used as E551.

[34]As reported by the authors.

[35]Calculated by the Panel: 500 mg/kg E 551 corresponding to 75 mg E551/SiO_2 considering a rat bw of 150 g; SiO_2 contains 46.7% Si, hence 75 mg SiO_2 contains $75 \times 0.467 = 35.02$ g Si.

[36]Reported by the authors as Aerosil® 200 which according to Appendix A of the EFSA ANS Panel (2018) is used as E551.

[37]Reported by the authors as Sipernat® 22 which according to Appendix A of the EFSA ANS Panel (2018) is used as E551.

is a strange and unexplained finding. The fate of E 551 was studied in the liver by TEM analysis on day 1, day 28 and day 29 in the highest dose group (2000 mg/kg bw for 28 days). The authors report that on day 28, in the liver, relatively intact particles were found for precipitated E 551 while particles from fumed E 551 were dissolved partially into ions. The particles were no more observed in the liver at 29 day. No particles were detected in the kidney, only ionic form resulting from decomposition of E 551 particles. EDX analysis was performed to identify the elemental composition of the particles. The results were interpreted by the authors as indicating the presence of Si in the livers of treated animals but not in the controls. The Panel considered the data on day 29 to be inconsistent with the other concentration measurements in the study.

In the study of van der Zande et al. (2014), E 551[38] and NM-202 were used. In the 28-day study, three groups ($n = 5$ each) of SD rats were fed E 551 at 83, 819, 2047 mg/kg bw per day; further three groups ($n = 5$ each) received NM-202 at 82, 405 and 810 mg/kg bw per day. One group ($n = 5$) served as control. Three groups of rats ($n = 5$ each) were studied for 84 days and given E 551 at 2500 mg/kg bw per day or NM-202 at 1000 mg/kg bw per day; one group served as control. Total Si content was determined with HDC-ICP-MS in the liver, kidney, spleen, brain and testis, and converted and reported by the authors as silica content. The LOD in tissues was 35 mg Si/kg (75 mg SiO_2/kg). SEM–EDX analysis of liver tissue was performed. No silica particles (5–200 nm) could be detected in the liver of exposed rats using SEM–EDX. After 28-days of exposure no significant increases of total silica were observed in the examined organs except for the animals administered with the lower dose of NM-202. After 84-days of exposure, the silica concentration in the spleen of animals that received the E 551 dose of 2500 mg/kg bw per day was significantly higher than in the controls. In all other treated groups and tissues, no significant increase compared with the control group was observed. In this study, the amount of silica (5–200 nm) in tissues from the exposed animals could not be determined nor was it possible to elucidate in which form silica was taken up. From this study, the Panel considered that the tested material (E 551, NM-202) is not absorbed/systemically available to a measurable extent when administered by the oral route.

In the Nanogenotox Project (De Jong et al., 2013) and the follow-up publication by Aureli et al. (2020), the kinetics of NM-200 and NM-203 were investigated in rats after oral administration by gavage of a single dose (20 mg/kg bw) and repeated doses (20 mg/kg bw on 5 consecutive days, total dose 100 mg/kg bw) followed by a recovery period of 14 days. Si concentration was measured by DRC-ICP- MS at different time points (6 day, 14 day). In female rats, following repeated oral administration of NM-200, similar Si concentrations could be measured in the liver on day 6 and day 14; in the spleen the concentration on day 14 was slightly higher than in the control and higher than on day 6, rendering the results questionable. The concentrations in the mesenteric lymph nodes of treated and control animals were below the LOD for both materials. In male rats, following repeated oral administration of NM-200 or NM-203, the Si concentrations were below the LOQ in the liver, spleen, and mesenteric lymph nodes at all time points. The small intestine contained measurable Si concentrations which were lower than the concentrations of the controls for all time points except on day 14. In both sexes, blood Si concentrations for both materials were below or just at the LOQ and lower than the concentrations in the controls. From this study, the Panel considered that the tested material (NM-200, NM-203) is not absorbed/systemically available to a measurable extent when administered by the oral route at a low dose of 100 mg/kg bw.

In the study of Kwon et al. (2024), fumed E 551[39] and precipitated E 551[40] were given to female SD rats at a dose of 2000 mg/kg bw by gavage for 28 days. Urine and faeces were collected for this time period and for additionally up to day 90. Particles were analysed by TEM-EDX. Silicic acid was measured in urine by the molybdenum blue complex analysis and by ICP-OES. The results show that between 76.5% and 96.8% of the total Si levels in urine is excreted after bioconversion as soluble silicic acid. The authors show that the amount of silicic acid excreted in the urine was zero on the first day after administration of E 551 was stopped (day 29). This finding is implausible, given that the half-life of Si is about 15 days. The percentage of the dose in the urine, claimed by the authors to be 3.1% and 3.9% for fumed and precipitated E 551, respectively, is not in accordance with the low systemic availability of less than 1%. The authors did not explain how they determined the percentage of the dose absorbed from the urinary excretion data. The Panel considered that the result may be due to faulty calculations or due to faecal contamination of the urine. The faecal excretion amounted to 97%–99% of the dose. The analysis by TEM led the authors to conclude that the statistical particle size distribution was the same in the faeces samples as in pristine material before administration.

In an unpublished 28-day oral toxicity study (Fraunhofer, 2011, documentation provided to EFSA No. 14), doses of 100, 300 and 1000 mg NM-200/kg bw per day were given to Wistar rats. The Si concentration in the blood, kidney and liver was measured by IPC-MS for the 1000 mg/kg bw per day group. The Si concentrations in the kidney, liver and blood were comparable to those in control animals. Two samples per organ of the mesenteric lymph nodes, kidney and liver were analysed by TEM in animals from the control and the highest dose group. In both examined groups, electron dense structures were found occasionally in the cytoplasm of all investigated tissues. These electron dense structures were noted in vacuoles and were characterised by irregular homogenous to fine granular material with a size of a few nanometres. The structures did not show the shape or appearance of amorphous material such as amorphous silica. The Panel concluded that the study showed that the material is only absorbed and systemically available to a very low extent.

In an unpublished study (AnaPath, 2020, Documentation provided to EFSA No. 10), Si distribution in organs and deposition tissue were examined from a previous two-generation reproductive toxicity study (TNO Triskelion, 2012; documentation

[38] As reported by the authors.

[39] Reported by the authors as Aerosil® 200 which according to Appendix A of the EFSA ANS Panel (2018) is used as E551.

[40] Reported by the authors as Sipernat® 22 which according to Appendix A of the EFSA ANS Panel (2018) is used as E551.

provided to EFSA No. 18) in Wistar rats with NM-200 at dose levels of 100, 300 and 1000 mg/kg bw per day. The doses were administered by gavage during a premating period of 10 weeks, during mating, gestation and lactation to the F0 animals. The analysis was conducted on adult F1-generation offspring that were dosed by gavage from postnatal day 22 until the day prior to sacrifice. Samples from all available epoxy resin-embedded organs of 2 control and 2 F1-animals from the group given 1000 mg/kg bw per day were used. The samples were analysed by SEM and EDX. EDX analysis was performed on every suspicious spot of the evaluated organs. A number of samples contained spots with a composition of Na, Al, and Si that are interpreted as Feldspar (Albit) and were clearly sticking between cells. In several cases, the particles observed were located free on the surface, and hence were deemed to be dust particles (contamination). Tiny 'silica crystals' were observed in single control organs (spleen, intestinal segments). In treated animals, such silicon particles were found at an increased incidence in the oesophagus and gastrointestinal tract. The authors concluded that they did not see an uptake of Si containing particles in the treated animals that is different from the controls.

Summary

Regarding the nanoscale considerations, the Panel noted that in vitro models and in vivo kinetic studies have been assigned scores of 1–4. Depending on the dispersion protocol before administration, animals have been exposed to small aggregates and large agglomerates. From in vitro studies the mechanism of absorption by the gastrointestinal tract is demonstrated to be energy-dependent endocytosis. Small particles were seen intracellularly in vesicles, and only in one study in the cell nucleus.

For the toxicokinetics, measured Si concentrations were used as surrogate for SAS particles to characterise the concentration in blood or tissues at different time points; in the available data set no study was identified that measured particles. The challenge of the Si measurement consists in the fact that the Si content of feed is high and variable, resulting in high background Si concentrations also in control animals and that technical difficulties exist. Hence, only in a few studies the Si concentrations were found to be higher in the treated animals than in the controls.

From intravenous studies in rats and mice, the Panel concluded that the half-life is about 15 days, indicating accumulation of about 90 days until steady state is reached.

From the few studies that could quantify the Si concentrations in tissues, the Panel concluded that the absorption/systemic availability of SAS is below 0.2% and that this percentage decreases with increasing dose. No direct information could be obtained on the percentage of systemic availability in relation to the particle size. However, from the reduced percentage of systemic availability at higher doses, it can be assumed that SAS at higher doses will be present mainly as larger agglomerates.

In urine, Si is found mainly in the form of silicic acid, indicating that systemically available SAS particles dissolve and that silicic acid is formed. In faeces, the excretion of SAS was quantified as being 97%–99%.

3.5.2 | Acute toxicity

Studies with duration of exposure less than 28 days were not considered for derivation of reference point and therefore not included in the current assessment.

3.5.3 | Short-term and subchronic toxicity studies

For the evaluation of the short term and subchronic toxicity potential of E 551, 11 studies were considered of sufficient internal validity (RoB 1 or 2). One study was unpublished (BSL Bioservice 2020 and 2023, documentation provided to EFSA No. 20). Two additional studies of lower internal validity (RoB 3) (Gmoshinski et al., 2020; Tassinari et al., 2020) have been considered as supportive information.

Four studies were conducted in rats (van der Zande et al., 2014; Liang et al., 2018; Badawy et al., 2023; BSL Bioservice 2020, 2023, documentation provided to EFSA No. 20) and seven studies were conducted in mice (Lamas et al., 2024; Li et al., 2018; Lu et al., 2021; Perez et al., 2021; Sofranko et al., 2022; Yan et al., 2020; Zhang et al., 2017).

With respect to the nano-specific considerations, two studies (Badawy et al., 2023; Zhang et al., 2017) were rated NSC 2; five studies (Lamas et al., 2024; Li et al., 2018; Lu et al., 2021; Perez et al., 2021; van der Zande et al., 2014) with NSC 3; and four studies (Sofranko et al., 2022; Liang et al., 2018; Yan et al., 2020; BSL Bioservice 2020 and 2023) with NSC 4.

The details of the toxicity studies are reported in tabular form in Annex G.

Studies in rats

In a 90-day repeated dose oral study (BSL Bioservice, 2020 and 2023, documentation provided to EFSA No. 20) (Annex G, Summary table G.1) (RoB 1/2; NSC 4), E 551[41] precipitated was administered daily by oral gavage at the dose of 1000 mg/kg bw per day to male and female Wistar rats (10/sex/group) for 90 days (endpoints in accordance with OECD TG 408,

[41] As reported by the authors.

(OECD, 2018b)). No toxicologically relevant findings were observed in any of the dose groups during the treatment or recovery period of the study. There were no treatment-related histopathological changes in any organ/tissue (including enhanced evaluation of thymus, lymph nodes, spleen, plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST), bone marrow and Peyer's patches). No adverse effects were observed with E 551 at the only dose tested of 1000 mg/kg bw per day.

In the study of Badawy et al. (2023), (Annex G, Summary table G.2) (RoB 1/2 for clinical signs, mortality, liver weight, serum ALT, serum AST, kidney weight, serum creatinine; RoB 3 for liver histopathology, kidney histopathology, adrenal weight and histopathology, serum cortisol; NSC 2) SAS was administered daily by gavage at a dose of 100 mg/kg bw to male Sprague–Dawley rats (6 males/group) for 90 days. A control group received water only. No clinical signs of toxicity and mortality were observed during the exposure time. Rats were sacrificed, and blood was collected for the estimation of the liver transaminases (ALT and AST), serum creatinine and cortisol values; the liver, kidneys and adrenal glands were excised for histopathology observation. The clinical chemistry provided no meaningful indications of adverse effects, whereas the histopathology was very limited and inadequate. No adverse effects were observed with SAS at the only dose tested of 100 mg/kg bw per day.

In the study of Van der Zande et al. (2014) (Annex G, Summary table G.5) (RoB 1/2, NSC 3), the test substances were administered via the diet at the dose of 83, 819 or 2047 mg E551[42]/kg bw per day, and 82, 405 or 810 mg NM-202/kg bw per day to male Sprague Dawley rats (5 males/group) for either 28 days (control and all doses) or 84 days (control and highest dose only). One control group was used for E 551 and NM-202.

At 28 days, no adverse effects were observed at all doses for both E 551 and NM-202. However, after 84 days of exposure at the dose of 810 mg/kg bw per day, the occurrence of periportal fibrosis was reported by the authors to be significantly increased in the NM-202 exposed animals ($p = 0.021$), as compared with the control animals. In liver homogenates obtained from animals treated for 84 days with NM-202, significantly induced expression of mRNA transcribed from a 'fibrosis-related gene set' was observed. The Panel noted that there was no measurable increase in liver Si compared to controls in rats treated with high dose NM-202 for 84 days and reported to develop liver fibrosis. Furthermore, there was minimal evidence of hepatocellular or biliary cell injury based on both histopathological and clinical chemistry endpoints. The Panel also noted an apparent 'very mild' or 'mild fibrosis' observed by the authors in three out of five control animals and considered that the set of fibrosis genes analysed in their microarray was limited in scope. The Panel noted a degree of connective tissue hyperplasia around the periportal vasculature in the absence of any parenchymal infiltration but considered the data presented to provide no evidence of periportal liver fibrosis at 84 days in rats treated with NM-202 at the highest dose level. Therefore, the 'fibrosis' effects reported by the authors are considered by the Panel not to be adverse.

The Panel considered that no adverse effects were observed after 84 days dietary treatment with NM-202 at the only dose tested of 810 mg/kg bw per day. The Panel noted that no adverse effects were observed after 84 days dietary treatment with E 551 at the only dose tested of 2047 mg/kg bw per day.

In the study of Liang et al. (2018) (Annex G, Summary table G.7) (RoB 1/2, NSC 4), precipitated SAS was administered by gavage at the doses of 0, 166.7, 500 or 1500 mg/kg bw per day to female Sprague–Dawley rats (5/female/group) for 90 days (endpoints according to OECD TG 408, (OECD, 2018b)). There were no toxicologically significant changes in mortality, clinical signs, body weight, food consumption, necropsy findings, organ weights, haematology and clinical chemistry. Focal epithelial cell proliferation and foreign body granulomas were found in the lungs of all silica exposed groups. The incidence and severity of these lesions were higher in all SAS groups compared to controls but not dose-related. The Panel considered that these findings were due to the gavage administration (Damsch et al., 2011). The authors concluded that SAS did not induce dose-related adverse effects after 90 days oral exposure in rats. The Panel agreed with the authors' conclusion and identified the NOAEL at 1500 mg/kg bw per day.

Studies in mice

In the study of Yan et al. (2020) (Annex G, Summary table G.4) (RoB 1/2, NSC 4), SAS was administered daily by gavage at the dose of 0, 25, 160 or 300 mg/kg bw per day in 0.9% saline vehicle to male mice (ICR, adult) (8/male/group) for 28 days. No adverse effects were reported in this study and therefore the Panel identified the NOAEL at 300 mg/kg bw per day.

In the study of Li et al. (2018) (Annex G, Summary table G.11) (RoB 1/2, NSC 3), Kun-Ming mice (15/male/group) were dosed by gavage daily for 30 days with vehicle (drinking water) or 10 mg SAS/kg bw per day. The authors reported statistically significantly increased hepatic inflammation and fibrosis (evaluated semi-quantitatively by microscopy), hepatic mRNA levels of pro-inflammatory cytokines IL-1β and TNF-α, hepatic malondialdehyde and 8-hydroxy-2-deoxyguanosine levels, apoptosis and a statistically significant decrease in catalase activity after the SAS exposure. The TEM of liver samples from the exposed mice demonstrated presence of SAS inside hepatic mitochondria. The authors concluded that the exposure to 10 mg SAS/kg bw per day induced liver injury manifested in 'inflammation infiltration' and fibrosis in mice. The Panel noted that (i) histopathological examination was performed in only four livers from each group and the incidences of lesions were not reported; (ii) although a statistically significant difference to the control was reported the severity of inflammatory infiltration was mild and of fibrosis between mild and moderate in SAS and below mild in the control group; (iii) foci of inflammatory cells in mouse liver occur spontaneously and (iv) fibrosis was diagnosed by the authors based on

[42]As reported by the authors.

the collagen presence around blood vessels demonstrated by Masson staining. In the light of the above, the Panel considered the effects reported by the authors as 'fibrosis', not to be adverse effects. No adverse effects were reported on survival, body weight, blood insulin and glucose levels, oral glucose tolerance, hepatic superoxide dismutase activity and relative liver weight. The Panel considered that no adverse effects were observed in mice receiving 10 mg SAS/kg bw per day by gavage for 30 days.

In the study of Lu et al. (2021) (Annex G, Summary table G.10) (RoB1/2, NSC 3), precipitated E 551[43] and fumed E 551[44] were administered in the diet at doses of 225, 1000 or 5000 mg/kg bw per day to male BALB/c mice for either 28 (6 males/group) or 84 days (7 males/group). The 28-day dietary exposure to precipitated E 551 or fumed E 551 had no effect on body weight, plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, relative weights and morphology of the heart, spleen, lungs, kidneys and brain up to 5000 mg/kg bw per day. A statistically significantly decreased relative liver weight at the mid-dose of fumed E 551 and mild steatosis in the liver of the mid- and high-dose precipitated E 551 or fumed E 551 groups were reported.

The 84-day exposure up to 5000 mg/kg bw precipitated E 551 or fumed E 551 had no effect on body weight. Statistically significant increases in activity of ALT at the high dose of precipitated E 551 and of AST at the low and high dose of precipitated E 551 (no dose dependency) were recorded. Myeloperoxidase (MPO) activity in the liver was statistically significantly increased at the high dose of precipitated E 551. No effect on organ weights except a statistically significant decrease in the relative liver weight at all doses of precipitated E 551 and the relative kidney weight at mid- and high- dose of fumed E 551. No histopathological changes were found in the heart, spleen and brain after exposure to doses up to 5000 mg/kg bw of precipitated E 551 or fumed E 551. Histopathological changes reported in the liver were steatosis at all three doses of precipitated E 551 and fumed E 551, and hepatocyte necrosis and infiltration with inflammatory cells at the high dose of precipitated E 551. In addition, lung inflammation and lymphocyte infiltration and connective tissue hyperplasia in the kidney were reported in the high-dose precipitated E 551 group. The internal exposure to silicon was confirmed by ICP-OES in major organs (liver, lung, kidney, spleen, stomach and intestines). The Panel noted that no information on incidence and severity of histopathological changes was provided in the publication (apart from a statement that liver steatosis was mild after 28-day exposure). The Panel identified the NOAEL at 1000 mg/kg bw per day.

In the study of Sofranko et al. (2022), (Annex G, Summary table G.9) (RoB 1/2 for non-neurotoxicity related endpoints, RoB 3 for functional neurotoxicity endpoints; NSC4), fumed SAS was administered in the diet at the dose of 0.1% or 1% (equivalent to 200 or 2000 mg SAS/kg bw per day) to female C57BL/6J mice (6/female/group) for 3 or 14 weeks. Fumed SAS had no effects on body weight, body weight gain, spatial working memory, agility and grip strength, and organ weight after 3 or 14 weeks, and on motor coordination and balance after 14 weeks. After 14 weeks, the mice in the 1% SAS group spent statistically significantly less time in the central region of the open field arena compared to the control mice, which indicated increased anxiety. No treatment-related histopathological changes were observed in the liver, spleen and small and large intestine after 14 weeks. The Panel identified a NOAEL at a dietary concentration of 1% SAS equivalent to 2000 mg/kg bw per day.

In the study of Zhang et al. (2017) (Annex G, Summary table G.3) (RoB1/2, NSC 2), male Kun Ming mice were dosed orally by gavage with 0 or 250 mg/kg bw per day of E 551[45] or S350 for 30 days. Because S350 is not a relevant material for this assessment, only the results obtained with E 551 were considered. A limited haematological investigation demonstrated no difference between the exposed group and the control in platelet, erythrocyte and leucocyte counts and in histopathological examination of the liver, heart, spleen lung, kidney and small intestine. An increase in granulocyte count (+182%) and in lymphocyte subtype CD4+ T helper cells (+13%) was reported in the E 551 group. The Panel noted that the changes in subtypes of leucocytes were observed in the absence of changes in the total number of leucocytes, i.e. with no adversity. Other immunological parameters have been assessed in Section 3.5.7.

In the study of Perez et al. (2021) (Annex G, Summary table G.8) (RoB 1/2, NSC 3) male mice received NM-202 at dietary concentration of 0, 0.8, 8 or 80 mg/kg diet equivalent to doses of 0, 0.14, 1.4, and 14 mg/kg bw per day for 24 weeks. Treatment had no effects on body weight, feed intake, survival, blood glucose homeostasis, caecal and serum concentration of lipocalin-2 (LCN-2) (a biomarker of intestinal inflammation), serum-amyloid-A1 (SAA-1) and IL-6 transcript levels (biomarkers of systemic inflammation), total cholesterol, morphology of the ileum and colon, or atheromatous plaque formation in the thoracic aorta. The changes in other endpoints (blood lipids, alanine aminotransferase, and urea) were considered not adverse. No adverse effects were observed at 14 mg/kg bw per day.

3.5.4 | Genotoxicity

The in vitro and in vivo genotoxicity studies have been assessed by the EFSA cross cutting (cc)WG Genotoxicity based on the available procedure on the approach for assessing reliability and relevance of genotoxicity studies (EFSA, 2023). Only studies of high and limited relevance have been included in the WoE. The detailed assessment is provided in the Annex B.

[43] As reported by the authors.

[44] Reported by the authors as Aerosil® 200 which according to Appendix A of the EFSA ANS Panel (2018) is used as E551.

[45] Reported by the authors as Aerosil® 200 which according to Appendix A of the EFSA ANS Panel (2018) is used as E551.

Gene mutations

In vitro

Precipitated SAS (NM-200) and fumed (E 551[46]) did not induce gene mutations in mouse lymphoma L5178Y/TK$^{+/-}$ or CHO cells with and without metabolic activation in two in vitro studies (Fraunhofer, 2012a, documentation provided to EFSA No. 15; Cabot et al., 1990b, documentation provided to EFSA No. 13). Similarly, negative results were also reported at the *HPRT* gene in V79 cells exposed to precipitated (NM-200) and fumed (NM-203) SAS (ICCR, 2024a, ICCR 2024b, documentation provided to EFSA No. 31 and 32).

In vivo

In vivo data are limited to two negative gavage studies: a dominant lethal assay performed in Sprague Dawley rats administered SAS gel E 551[47] (Litton Bionetics, 1974, documentation provided to EFSA No. 1) and a gene mutation test at the Pig-A gene in red blood cells and reticulocytes of Sprague–Dawley male rats administered fumed SAS (NM-203) (Villani et al., 2022).

DNA damage

In vitro

Precipitated SAS (NM-200) did not induce any increase of DNA damage in primary rat alveolar macrophages from bronchoalveolar lavage (Fraunhofer, 2012a, documentation provided to EFSA No. 15) and in the human monocytic cell line THP-1 (Brzicova et al., 2019), whereas it produced contrasting results in the human bronchial epithelial cells BEAS-2B (negative in Zijno et al., 2020 and positive in García-Rodríguez et al., 2019) and in the human lung adenocarcinoma cells A549 (negative El Yamani et al., 2022 and positive García-Rodríguez et al., 2019). Negative results were also observed with precipitated SAS (NM-201 and NM-204) and fumed SAS (NM-202) in A549 cell line (El Yamani et al., 2022).

The fumed SAS (NM-203) resulted positive in many studies with different cell lines. Positive results were observed in BEAS-2B cells (García-Rodríguez et al., 2019; Zijno et al., 2020) and in A549 cells after a short exposure (El Yamani et al., 2022; García-Rodríguez et al., 2019; Precupas et al., 2020), while contrasting results have been reported by the same authors after longer exposure in the same cell line (A549). Gehrke et al. (2012) reported negative results for NM-203 in the human colorectal adenocarcinoma cells HT29 after 24 h of treatment. Most studies yielded negative results when the Fpg-modified comet assay protocol was used for oxidised bases detection.

E 551 (precipitated) produced negative results in intestinal cells HT29-MTX-E12 and Caco-2/HT29-MTX co-culture as reported by Franz et al. (2020) and Dussert et al. (2020), respectively.

Negative results were also reported for other SAS in V79 cells (Guichard et al., 2016), Caco-2/HT29-MTX co-culture (Dussert et al., 2020), rat alveolar type II epithelial cells (Brandão et al., 2023) and in A549 and BEAS-2B cells by Kain et al. (2012).

In contrast, positive results were observed with other SAS. Fumed SAS resulted positive in the mouse macrophage-like RAW264.7 cell line (Dussert et al., 2020) and in rat alveolar type II epithelial cells (Brandão et al., 2023). Three different SAS (of different constituent size: 10, 25 and 50 nm) increased DNA damage in HUVECs (Zhou et al., 2019). Two other SAS (30 or 15 nm size) produced a dose and size dependent increase in DNA damage in human epidermal keratinocyte cells (HaCaT) (Gong et al., 2012). Positive results were also obtained in mouse embryonic fibroblasts cells L929 (Alkahtane, 2015) and in Chinese hamster lung (CHL) cells (Lee et al., 2020).

NM-203 was reported negative in bronchoalveolar lavage fluid (BALF), lung, blood, bone marrow, liver, spleen, kidney of male Sprague Dawley rats treated by intravenous injection at 0, 3, 24 and 48 h (Guichard et al., 2015). NM-200, NM-201, NM-202 and NM-203 produced negative results for the induction of DNA damage in blood, bone marrow, liver, spleen, kidney, duodenum, colon of Sprague–Dawley adult rats administered by gavage at 0, 24 and 45 h, in a study by Tarantini et al. (2015) to which limited relevance of the result was assigned.

NM-203 was tested in Sprague–Dawley adult rats (both sexes) administered by gavage for 90 days: positive effects were reported in spleen (both males and females), while negative results were obtained in blood, bone marrow and liver (Villani et al., 2022) and in male and female reproductive tissues (Tassinari et al., 2021).

Chromosomal damage

Negative results were consistently observed in studies performed with fumed E 551, NM 200 and another SAS (for the induction of structural chromosome aberrations in CHO, V79 and CHL cell lines (Cabot et al., 1990a, documentation provided to EFSA No. 12; Fraunhofer, 2012b, documentation provided to EFSA No. 16; Lee et al., 2020).

No induction of micronuclei by NM-203 was observed in human bronchial BEAS-2B cells (Zijno et al., 2020), while contrasting results were reported for NM-200 by Zijno et al. (2020) and Cervena et al. (2021). Negative results were obtained for

[46]Reported by the authors as Cab-O-Sil EH5 which according to Appendix A of the EFSA ANS Panel (2018) is used as E551.

[47]Reported by the authors as Syloid 244 which according to Appendix A of the EFSA ANS Panel (2018) is used as E551.

NM-200, NM-201, NM-202 and NM-203 in human lymphocytes (Tavares et al., 2014) and other fumed and precipitated SAS in V79 cell line (Guichard et al., 2016).

In contrast, positive results were reported for the induction of micronuclei in undifferentiated intestinal Caco-2 cells treated with fumed SAS (NM-202, NM-203) and precipitated SAS (NM-200, NM-201) (Nanogenotox, 2012, documentation provided to EFSA No. 19). Similarly, SAS (of different constituent size: 12 nm, 5–10 nm, and 10–15 nm) increased the frequency of micronuclei at high level of cytotoxicity in mouse lung epithelial (FE1) cells derived from Muta™Mouse (Decan et al., 2016). Micronuclei were increased in human umbilical vein endothelial cells (HUVECs) treated with other SAS (of different constituent size: 10, 25 and 50 nm) with a negative size-dependent effect relationship (50 < 25 < 10) associated with an increase in ROS (Zhou et al., 2019).

Negative results for the induction of micronuclei in bone marrow erythrocytes were obtained in rats administered by gavage with SAS gel E 551 (Litton Bionetics, 1974, documentation provided to EFSA No. 1), NM-200 (Tarantini et al., 2015) and NM-203 (Villani et al., 2022), while equivocal results were reported for NM-202 and NM-203 at a single, low dose (Tarantini et al., 2015). A dose-related increase in the frequency of micronuclei in bone marrow was observed in rats after i.v. treatment with NM-203 (Guichard et al., 2015). Taking into account the toxicokinetics data, the results obtained after i.v. exposure are considered to have limited relevance for oral exposure.

Overall assessment

Data for all the relevant genotoxicity endpoints were available, but not for each type of SAS used as E 551. Since all the materials tested are relevant to the assessment of the food additive, all the available evidence on the different SAS materials was considered to conclude on the genotoxic potential of E 551.

The results of available in vitro and in vivo studies do not raise a concern regarding the induction of gene mutations by E 551 (fumed and gel), NM-200 and NM-203.

In vitro studies yielded mixed results regarding chromosomal damage, with no correlation found between this induction and certain experimental variables (e.g. cell type and type of SAS and specific experimental conditions). Conversely, in vivo studies with E 551 (gel), NM-200, NM-201, NM-202 and NM-203 reported negative outcomes for chromosomal damage after oral exposure. One positive result was obtained after i.v. exposure with NM-203, which is considered of limited relevance for oral exposure.

Both positive and negative results were reported for DNA damage induction in in vitro comet assays after exposure to E 551 (precipitated), NM-200, NM-203 and other SAS. In vivo, negative results were reported for NM-200, NM-201, NM-202 and NM-203 after short-term oral exposure. One positive result of limited relevance was reported in spleen for NM-203 after 90-day oral exposure, while negative results were observed in the same tissue after short treatments.

Regarding NSC, considering that the majority of in vitro and in vivo studies included in the present assessment have scores 1 or 2, and that the pattern of positive and negative results does not change significantly when considering the results all together (NSC 1–4) and separately for NSC 1–2. The Panel considered that the conclusion on the genotoxicity is applicable to nano aggregates present in E551. Overall, based on the currently available data set, the Panel concluded that E 551 does not raise a concern for genotoxicity.

3.5.5 | Chronic toxicity

One unpublished study was considered relevant (BSL Bioservice, 2021, Documentation provided to EFSA No. 4) for the evaluation of the chronic toxicity potential of E 551.

The details of the study have been reported in tabular form in Annex G, Summary table G.12.

In this GLP-compliant 12-month repeated dose oral chronic toxicity study conducted according to OECD Test Guideline No. 452 (OECD, 2018c) (RoB 1/2, NSC 4), E 551[48] was administered daily by gavage at the doses of 100, 300 or 1000 mg/kg bw per day to male and female Wistar rats [Crl:WI(Han)] (20/sex/group) for 12 months. Furthermore, a recovery control and a high-dose group were included in the study each comprising five males and five females and terminated 28 days after the end of the treatment.

Six female rats died or were prematurely euthanized in moribund condition, four of them in the high dose group. All deaths were considered related to the fact that the test item after dispersion was highly viscous and that administration resulted in stress and occasionally in misdosing.

No toxicologically relevant clinical signs or effects on ophthalmology, body weight, food consumption, parameters of haematology, coagulation, clinical chemistry and urinalysis were observed at the end of the treatment or recovery period. Statistically significant differences, mostly small, and considered not adverse, were not consistent across gender, dose or time period for any endpoint, and are therefore considered to be incidental, not related to treatment.

No compound-related gross findings or effects on organ weights were observed at necropsy. The histopathological examination of organ/tissues from the controls and the high-dose group (in accordance with the OECD test guideline) did not reveal any treatment-related changes.

[48]As reported by the authors.

There were also no specific findings such as fibrosis in the liver or kidneys. Furthermore, there were no lesions in immune organs (spleen, thymus, lymph nodes, ALT, ALT or bone marrow) that could be attributed to treatment with the test item. Based on the results of this 12-month study the Panel considered 1000 mg/kg bw per day, the highest dose tested, as the NOAEL.

3.5.6 | Reproductive and developmental toxicity

Three studies were identified as relevant (Fraunhofer, 2011, documentation provided to EFSA No. 14; Hofmann et al., 2015; TNO Triskelion, 2012, documentation provided to EFSA No. 18) for the evaluation of the reproductive and developmental toxicity potential of E 551, two of which were unpublished (Fraunhofer, 2011; TNO Triskelion, 2012). All studies were conducted in rats and considered reliable (RoB1/2). With respect to the nano-specific considerations, two studies were rated NSC 4 (Fraunhofer, 2011; TNO Triskelion, 2012) and one NSC3 (Hofmann et al., 2015).

The details of the toxicity studies have been reported in tabular form in Annex G.

One unpublished study report (TNO Triskelion, 2012, documentation provided to EFSA No. 18) was subsequently published in summarised form by Wolterbeek et al. (2015).

In the GLP-compliant two-generation reproductive toxicity study (TNO Triskelion, 2012, Annex G, Summary table G.15) (RoB 1/2; NSC 4) performed according to OECD TG 416 (OECD, 2001a), Wistar rats ($n = 28$/sex/group) were dosed daily by gavage with NM-200 in 0.5% aqueous methylpropylcellulose suspension at doses of 0, 100, 300 or 1000 mg/kg bw per day. There was no parental toxicity at any dose and no adverse effects on reproductive performance or on growth and development of the offspring into adulthood for two consecutive generations.

In the pathology report (AnaPath, 2020, documentation provided to EFSA No. 10), of a subsequent investigation of additional organs of the two-generation study (TNO Triskelion, 2012, documentation provided to EFSA No. 18), reported brain histopathology and morphometry and spleen histopathology endpoints measured in a subset of control and high dose F1 animals ($n = 10$/sex/dose). Si organ distribution was evaluated by EDX analysis in plastic embedded material from F1 females (the only available material). Intestine (plastic embedded material) was evaluated for CD11, CD40, CD69, CD86, IL1-beta and IL6-positive structures, as well as the measurement of villi length. F1 brains (10 rats/sex of control and high-dose groups) were examined histopathologically and by image analysis according to Garman et al. (2016). Spleen and thymus sections (if available) from these animals underwent enhanced immune system evaluation according to Elmore (2006a, 2006b). Concerning developmental neurotoxicity, the report states that there were no histological lesions in the brain sections in any control or high-dose animal, and no statistically significant differences in brain structure dimensions between control and high dose animals. The pathology report concluded that there were no treatment-related changes in the organs examined, no fibrosis in the liver, no change in the lengths of intestinal villi, no 'silicon particles' in any tissue other than on the surface of gastrointestinal tract, no brain pathology and no immune toxicity in spleen and thymus.

Histopathological investigation was done only on high-dose group and not on mid- and low-dose groups, which is in accordance with OECD test guidelines, however considering that nano aggregates are present in E 551, the Panel would have welcomed the analysis of all the dose groups. Milk samples were collected from animals of the two-generation reproductive toxicity study (TNO Triskelion, 2012 documentation provided to EFSA No. 18) and analysed for the presence of NM-200 particles by TEM/EDX (AnaPath, 2024, documentation provided to EFSA No. 29). No particles were seen. While the Panel acknowledged the difficulty to perform this kind of analysis, the method used for the analysis was considered not suitable to detect particles at low levels and, therefore, their presence cannot be excluded.

In a 28-day oral toxicity study (Fraunhofer, 2011, documentation provided to EFSA No. 14, Annex G, Summary Table G.13) (RoB 1/2, NSC 3), NM-200 was administered daily by gavage at the doses of 0, 100, 300 or 1000 mg/kg bw per day to male Wistar rats (5 males/group) for 28-days (OECD 407, 2008). NM-200 was suspended in 0.5% methylhydroxycellulose. The study included an extra control and high dose as recovery groups. No statistically significant effects were detected in testis and epididymis weight. Histology of testis, epididymis and seminal vesicles including coagulating glands of the control and high-dose groups showed no adverse effects. The NOAEL of the study based on these findings was 1000 mg/kg bw per day.

In the study of Hofmann et al. (2015) (Annex G, Summary table G.14) (RoB 1/2, NSC 3) (GLP, OECD TG 414 adopted in 2001 (OECD, 2001b), NM-200 was administered daily by gavage at doses of 0, 100, 300 or 1000 mg/kg bw per day to female Wistar rats (25/group) from gestation day 6 to 19. There were no adverse maternal or developmental effects up to the highest dose of 1000 mg/kg bw per day. The Panel identified the NOAEL as 1000 mg/kg bw per day.

Based on the results of all reported developmental and reproductive toxicity studies, including neuro- and immune system toxicity, the Panel identified a NOAEL for reproductive and developmental toxicity at 1000 mg/kg bw per day.

3.5.7 | Immunotoxicity

Thirteen publications were identified as relevant for the evaluation of the immunotoxic potential of E 551: four were unpublished studies (Fraunhofer, 2011 – documentation provided to EFSA No. 14; BSL Bioservice, 2021 – documentation provided to EFSA No. 4; AnaPath 2020 – documentation provided to EFSA No. 10; BSL Bioservice,2020 and 2023 – documentation provided to EFSA No. 20) the remaining were identified from the published literature (Gmoshinski et al., 2020;

Lamas et al., 2024; Liang et al., 2018; Lu et al., 2021; Sofranko et al., 2022; Tassinari et al., 2020; van der Zande et al., 2014; Yan et al., 2020; Zhang et al., 2017). All publications were considered reliable (RoB 1/2) with the exception of the publication by Tassinari et al., 2020 of lower reliability (RoB 3). The publication of Gmoshinski et al., 2020 reports two studies: The first experiment was scored RoB 3 due to changes in way of administrations (during the first 30 days, SAS was administered by gavage, and during the subsequent 62 days, SAS suspension was added to animal feed). In addition, randomisation is not mentioned, and statistical analysis Student's *t*-test is not appropriate for dose-related experiments. The second experiment was scored RoB 2. The publication by Lamas et al. (2024) reports four animal studies: three were considered RoB 1 whereas one was scored RoB 4.

The publication of Gmoshinski et al. (2020) and Lamas et al. (2024) are described in Section 3.5.8.

Five publications reported experiments conducted in mice (Lamas et al., 2024; Lu et al., 2021; Sofranko et al., 2022; Yan et al., 2020; Zhang et al., 2017) and seven in rats (Fraunhofer, 2011; van der Zande et al., 2014; Liang et al., 2018; Gmoshinski et al., 2020; AnaPath 2020; BSL Bioservice, 2021; BSL Bioservice, 2020 and 2023).

With respect to the nano-specific considerations, only the study by Tassinari et al. (2020) (RoB 3) was rated NSC 1, and the study by Gmoshinski et al. (2020) was given NSC 2. The studies by Van der Zande et al. (2014); Lu et al. (2021); Lamas et al. (2024) were scored NSC 3, while all the other studies (Fraunhofer, 2011; Liang et al., 2018; Yan et al., 2020; BSL Bioservice, 2021; Sofranko et al., 2022; AnaPath 2020; BSL Bioservice, 2020 and 2023) were given NSC 4.

In the different studies, several parameters related to immune system status or functionality were investigated: blood total leucocytes and differential count, lymphocyte subpopulations, spleen and thymus weights and histology, lymph nodes (mesenteric, mandibular, axillary, cervical) weights and histology, cytokine production, serum C-reactive protein, serum immunoglobulin, B- and T-cell proliferation, NK cell activity, peritoneal macrophage functions, colon cytokine immunohistochemistry. Only two publications investigated apical endpoints related to immune-mediated disorders (Gmoshinski et al., 2020; Lamas et al., 2024), which are described in Section 3.5.8.

Three studies conducted in male KM mice (RoB 2, Zhang et al., 2017, Annex G, Summary table G.3), ICR male mice (RoB 2, Yan et al., 2020, Annex G, Summary table G.4) and in male C57BL/6J (RoB 1, Lamas et al., 2024, Annex G, Summary table G.17) suggest a possible dose-related pro-inflammatory effect as assessed by pro-inflammatory cytokines at doses ≥ 160 mg/kg bw (Zhang et al., 2017), at 250 mg/kg bw (Yan et al., 2020) or at 10 mg/kg bw (Lamas et al., 2024). In all studies, however, signs of inflammation were not associated with adverse histopathological effects in the heart, liver, spleen, lung, kidney or small intestine after 1 month of treatment up to 300 mg/kg or changes in intestinal permeability up to 100 mg/kg bw.

Two publications, van der Zande et al. (2014) (Annex G, Summary table G.5) and AnaPath 2020 (Annex G, Summary table G.15) (both RoB 1/2), showed no immunotoxic effect in rats up to 2500 mg/kg bw. Two other publications (Gmoshinski et al., 2020; Tassinari et al., 2020), with RoB 3 and 2, respectively, reported sex-dependent effects on immunological parameters (Tassinari et al., 2020), serum cytokines and changes in Th subpopulations (Gmoshinski et al., 2020), which however, were not associated with abnormal clinical signs or with increased severity of anaphylactic reaction.

Considering the limited number of studies, the different doses used, and the different immunological parameters evaluated, inconsistent among the different studies, it is very difficult to establish or not a potential immunotoxicity of SAS. The most comprehensive immunotoxicity study was conducted in rats using high doses of SAS and NM-202 (van der Zande et al., 2014), and no effects were observed in any of the parameters investigated. Under some experimental conditions, SAS may induce pro-inflammatory effects (Gmoshinski et al., 2020; Yan et al., 2020; Zhang et al., 2017), which were not associated with abnormal clinical signs in the investigated models. Cytokines or changes in lymphocyte subpopulations are intermediate parameters are involved in all physiological and pathological immune responses, and there are limited number of publications ($n = 2$) addressing apical endpoints (Gmoshinski et al., 2020; Lamas et al., 2024, see below 3.5.8).

3.5.8 | Hypersensitivity, allergenicity and food intolerance

In the current assessment, two publications (Gmoshinski et al., 2020; Lamas et al., 2024) investigated food allergenicity, food intolerance and gluten-induced immunopathology in experimental animals. In both publications, more than one animal study was conducted, for which different RoBs were identified as specified below.

In Gmoshinski et al. (2020), two studies were conducted, one of which is relevant for this section (RoB1/2 assigned to the 28 day study, RoB 3 assigned to the 92-day study; NSC 2) (Annex G, Summary table G.16). In this study, the effect of 100 mg/kg bw SAS, administered by gavage for 28 days, on ovalbumin-induced systemic anaphylaxis reaction was investigated in Wistar rats. In animals parenterally immunised with the food allergen ovalbumin (OVA), SAS had no significant effect on the severity of the anaphylactic reaction or on the serum levels of specific IgG antibodies towards OVA.

The other publication (Lamas et al., 2024, NSC 3) (Annex G, Summary table G.17) included four studies, of which three are relevant for this section. Two studies investigated the effects of E 551[49] on oral tolerance to OVA (RoB 1/2) in C57BL/6J mice, and one investigated the effects on gluten sensitivity in autoimmune prone NOD/DQ8 mice (RoB 3). In the first study, C57BL/6J mice were daily treated for 60 days by gavage with E 551 (1, 10, 100 mg/kg bw per day) and were then tested for oral tolerance to OVA. Results indicate a treatment-related breakdown of oral tolerance; there were statistically significant increased levels of serum Ig anti-OVA at all doses; increased faecal lipocalin-2 at 10 and 100 mg/kg bw per day; increased

[49] As reported by the authors.

mesenteric-derived IFN-γ at 10 mg/kg bw per day, and colon IL-1β at all doses; decreased mesenteric IL-10, TGF-β, and colon IL-10, TGF-β mRNA; decreased colon TGF-β at all doses. The only clearly dose-related effect was observed for faecal lipocalin-2. In the second study, oral tolerance to OVA was investigated in C57BL/6J mice exposed to E 551 at 10 mg/kg bw via either gavage or in feed. Similar results as in the first study were obtained for both gavage and feed administration, indicating that increased intestinal inflammation after oral OVA challenge in mice exposed to E 551 was independent of food matrix. The only notable difference between gavage and feed exposure was a reduction of CD45 + CD11b + CD103+ cells (which include dendritic cell and macrophage subpopulations) in colon lamina propria, observed only in mice exposed to E 551 by gavage. Authors speculate that this difference is possibly due to the binding of food components on the NPs forming a protein/biomolecule corona, which could affect interaction with immune cells. The third study was conducted in NOD/DQ8 mice, a transgenic model used to study autoimmunity. These non-obese diabetic (NOD) mice exhibit a susceptibility to spontaneous development of autoimmune insulin-dependent diabetes mellitus, and other autoimmune syndromes, including autoimmune sialitis, autoimmune thyroiditis and autoimmune peripheral polyneuropathy. The HLA-DQ8 allele present in these transgenic mice also predisposes to several autoimmune diseases, including coeliac disease. In this specific study, the NOD/DQ8 mice were used to test gluten response. Mice were dosed by gavage daily for 75 days with E 551 (10 mg/kg bw per day) and previously sensitised with gliadin, the major component of wheat gluten. Results show increased gluten immunopathology in E 551-exposed NOD/DQ8 mice, raising the possibility that exposure to E 551 could act as a hypersensitising environmental trigger in genetically susceptible people consuming gluten.

3.5.9 | Human data

Case report

Only one case report on the occurrence of renal calculi containing silica was retrieved in the literature search.

A 38-year-old female patient was diagnosed with urinary gravel and small silicate stones in the urine as the cause for flank pain (Flythe et al., 2009). The patient took supplements, resulting in exposure to silica up to 0.96 mg/kg bw per day. After stopping the intake, no further symptoms were noted. When the patient resumed the intake of the supplements, the symptoms returned. Because the daily dose was far below doses assumed to be safe, the authors discuss possible contributing factors, among them unknown sources of exposure. The authors give references of reports on kidney stones of silica dating back to the 1960s, mostly seen in conjunction with the continuous use of antacids containing silica or in Japan with the use of drinking water with a high concentration of silica.

The Panel assessed the case report based on the criteria of the WHO-UMC system (Edwards & Biriell, 1994). For the above-mentioned case report, based on these criteria, silica was identified as the underlying cause the formation of silica urinary gravel and stones.

However, similar cases of renal calculi containing silica in conjunction with the intake of the food additive E 551 have not been found in the literature search.

3.5.10 | Supporting evidence

As indicated in the Methodology section (see Section 2.2), studies with RoB 3 have been used as supporting evidence in selected cases with a justification. Several publications have been used to support evidence coming from studies RoB 1/2: Tassinari et al. (2020); Gmoshinski et al. (2020); Badawy et al. (2023) and Sofranko et al. (2022).

A summary of Tassinari et al. (2020) and the 90-day study in Gmoshinski et al. (2020) publication is given in Annex H.

In the Gmoshinski et al. (2020) publication, the 28-day study was considered of RoB 1/2; therefore, it is summarised in Section 3.5.8, 'Hypersensitivity, allergenicity and food intolerance'. In the publication of Badawy et al. (2023) and Sofranko et al. (2022), some endpoints were considered RoB 1/2; therefore, these endpoints are summarised in Section 3.5.3, 'Short-term and subchronic toxicity studies'; while other endpoints were considered RoB 3.

3.5.11 | Weighting the body of evidence

In order to perform the WoE, the Panel grouped the studies according to the target organ/system and the related endpoints (see Annex D). In a first step, all studies considered of higher reliability (RoB1/2) were included in the WoE regardless of their score with respect to the nanoscale considerations. The assessment of the overall body of evidence was conducted qualitatively by expert judgement.

In a second step, and whenever the data allowed to do so, the outcome of the studies rated as having the higher level of confidence that nano-sized particles/aggregates were tested under the experimental conditions (NSC 1 and NSC 2) was compared with the outcome of the studies rated of lower confidence with respect to nanoscale considerations (NSC 3 or 4), to verify whether there could be specific findings related to the exposure to nano-sized particles/aggregates compared to large agglomerates (Table 9).

TABLE 9 List of the toxicity studies included in the WoE according to the target organ/system, the internal validity score (RoB score) and the nanoscale consideration score (NSC).

Author/year	Target organ/system	RoB score	NSC
Badawy et al. (2023)	Liver	1 or 2 for weight and ALT AST 3 for histopathology	2
	Kidney	1 or 2 for weight and creatinine 3 for histopathology	
	Adrenals	3	
	General toxicity	1 or 2	
BSL Bioservice (2020 and 2023)	Liver, kidney, thyroid and pituitary gland, bones, lungs, adrenals, reproductive system, immune system, gastrointestinal system, nervous system, general toxicity, endogenous metabolism, vascular toxicity	1 or 2	4
Zhang et al. (2017)	Liver, kidney, immune system, gastrointestinal system, general toxicity, vascular toxicity	1 or 2	2
Yan et al. (2020)	Liver, kidney, immune system, gastrointestinal system, general toxicity, vascular toxicity, endogenous metabolism	1 or 2	4
Van der Zande et al. (2014)	Liver, kidney, immune system, reproductive system, gastrointestinal system, nervous system, endogenous metabolism, general toxicity	1 or 2	3
Perez et al. (2021)	General toxicity, liver, endogenous metabolism, vascular toxicity	1 or 2	3
Gmoshinski et al. (2020)	Liver, kidney, adrenals, reproductive system, general toxicity, vascular toxicity	3	2
	Immune system	1 or 2	
Liang et al. (2018)	Liver, kidney, thyroid and pituitary gland, adrenals, reproductive system, immune system, gastrointestinal system, nervous system, general toxicity, endogenous metabolism, vascular toxicity	1 or 2	4
Fraunhofer (2011)	Liver, kidney, pancreas, thyroid and pituitary gland, bones, adrenals, reproductive system, immune system, gastrointestinal system, nervous system, general toxicity, endogenous metabolism, vascular toxicity	1 or 2	4
Sofranko et al. (2022)	Liver, immune system, gastrointestinal system, general toxicity	1 or 2	4
	Nervous system	3	
Lu et al. (2021)	Liver, kidney, immune system, nervous system, general toxicity	1 or 2	3
Li et al. (2018)	Liver, general toxicity	1 or 2	3
BSL Bioservice (2021)	Liver, kidney, pancreas, thyroid and pituitary gland, bones, adrenals, reproductive system, immune system, gastrointestinal system, nervous system, general toxicity, endogenous metabolism, vascular toxicity	1 or 2	4
TNO Triskelion (2012)	Liver, kidney, pancreas, thyroid and pituitary gland, adrenals, reproductive system, immune system, nervous system, developmental toxicity, general toxicity	1 or 2	4
Hofmann et al. (2015)	Reproductive system, developmental toxicity, general toxicity	1 or 2	3
Lamas et al. (2024)	Immune system	1 or 2	3

General toxicity

In this WoE analysis for general toxicity, the Panel assessed a total of 13 studies of different durations (28 days up to 1 year); eight studies were conducted in rats and five in mice. Regarding the nanoscale considerations, two studies were scored NSC 2, five studies were scored NSC 3 and six studies were scored NSC 4. The Panel considered the data set sufficient to assess the potential general toxicity in relation to the exposure of the tested animals to aggregates in the nano-range size and suitable for the exposure to large agglomerates.

The data set included the following parameters: body weight (10 studies), clinical signs (7 studies), haematology (5 studies), mortality (6 studies) and clinical chemistry (4 studies).

No study reported any adverse effects for general toxicity-related endpoints.

In a second step, the Panel considered the nanoscale scoring in the assessment. One study with RoB 3 and NSC 1, and 1 study RoB 3 and NSC 2 supported no effects on general toxicity. The Panel did not identify any differences in the outcome between studies with nanoscale score 2 vs. 3 and 4.

Based on the available data, the Panel concluded that it is likely that E 551 has no adverse effects on general toxicity endpoints.

Liver toxicity

In this WoE analysis for liver toxicity, the Panel assessed 13 studies of different durations (28 days up to 1 year); seven studies were conducted in rats and six in mice. Regarding the nanoscale considerations, there was no reliable study with NSC 1; whereas two studies scored NSC 2, four studies scored NSC 3 and seven studies scored NSC 4. The Panel considered the data set sufficient to assess potential liver toxicity in relation to the exposure of the tested animals to aggregates in the nano-range size and suitable for large agglomerates.

The data set included the following parameters: liver weight (10 studies), histopathology of the liver (10 studies), serum ALT and AST levels (8 studies).

In the studies in rats, no adverse effects were seen based on serum ALT, serum AST, serum albumin, total serum protein, serum bilirubin and total serum bile acids. No changes in liver weight and liver histopathology were observed. In one study in mice (NSC 3), decreased liver weight, mild steatosis and increases in serum ALT and AST levels were observed. The other studies in mice were not indicative for adverse effects on the liver. Based on WoE, the Panel considered there was no consistent evidence for adverse liver effects, also among the limited number of studies with NSC 2.

In a second step, the Panel integrated the nanoscale scoring in the assessment. One study with RoB 3 and NSC 1 supported no effects on liver. The Panel did not identify any differences in the outcome between studies with nanoscale score 2 vs. 3 and 4.

Based on the available data, the Panel concluded that it is likely that E 551 has no adverse effect on the liver.

Kidney and urinary tract toxicity

In this WoE analysis for kidney and urinary tract toxicity, the Panel assessed 10 studies of different durations (28 days up to 1 year) all scoring 1 or 2 for the internal validity (RoB); seven studies were conducted in rats and three in mice. Regarding the nanoscale considerations, there was no study that had NSC 1. Two studies scored NSC 2, two studies scored NSC 3 and six studies scored NSC 4. The Panel considered the data set sufficient to investigate potential kidney and urinary tract toxicity in relation to the exposure of the tested animals to aggregates in the nano-range size and suitable for large agglomerates of E 551.

The data set included the following parameters: kidney weight (8 studies), histopathology of the kidney (8 studies), serum creatinine (6 studies) and urea (4 studies) levels, urinary bladder and ureter histopathology (4 studies).

In the studies in rats, no adverse effects were found in serum creatinine and urea levels, or urinary bladder and ureter histopathology. These parameters were not measured in mice studies.

In one study in mice, with NSC 3, a decreased kidney weight was associated with exposure to E 551 at mid and high doses. In addition, lymphocytic infiltration and hyperplasia of connective tissue of the kidney was found. In one study with NSC 2, an increase of absolute kidney weight was associated with exposure to 100 mg SAS/kg bw per day (single dose). The Panel had low confidence that these findings represent adverse effects of E 551.

In a second step, the Panel integrated the nanoscale scoring in the assessment of kidney and urinary tract toxicity. One study with RoB 3 and NSC 1 and one study with RoB 3 and NSC 2 provided evidence of no effects on kidney and urinary tract. The Panel did not identify any differences in the outcome between studies with NSC 2 vs. 3 and 4.

WoE indicates that changes in kidney weight (absolute or relative) and histopathology did not occur across all studies. WoE consideration indicates that there is no consistent evidence for other potential adverse kidney effects. In studies in rats, no adverse effects were seen. Based on the available data, the Panel concluded that it is likely that E 551 has no adverse effect on the kidney and urinary tract.

Adrenal toxicity

In this WoE analysis for adrenal toxicity, the Panel assessed five rat studies of different durations (28 days up to 1 year) all scoring 1 or 2 for the internal validity (RoB); all studies scored NSC 4. The Panel considered the data set suitable to investigate potential adrenal toxicity in relation to the exposure of the tested animals to large agglomerates (NSC 4).

The data set included the following parameters: adrenal weight (5 studies) and histopathology (4 studies).

No evidence of adverse effects in terms of adrenal histopathology and adrenal weight was found.

In a second step, the Panel considered the nanoscale scoring in the assessment adrenals toxicity. Three studies with RoB 3 and NSC 1 and 2 provided evidence of no effects on these endpoints.

Due to the lack of reliable studies with NSC 1 and 2, comparison of the outcome of studies NSC 3 and 4 with that of NSC 1 and 2 was not possible.

Based on the available data, the Panel concluded that it is likely that E 551 has no adverse effect on the adrenals.

Thyroid and parathyroid toxicity

In this WoE analysis for thyroid and parathyroid toxicity, the Panel assessed five rat studies of different durations (28 days up to 1 year) all scoring 1 or 2 for the internal validity (RoB); all studies were given NSC 4. The Panel considered the data set suitable to investigate potential thyroid and parathyroid toxicity in relation to the exposure of the tested animals to large agglomerates (NSC 4), but the aggregates in the nano-range size were not tested.

The data set included the following parameters: thyroid and parathyroid weight (3 studies), thyroid and parathyroid histopathology (4 studies), pituitary gland weight (2 studies) and histopathology (5 studies), and thyroid hormones (T3, T4, TSH) (3 studies).

No adverse effects were found in the thyroid, parathyroid and pituitary gland (weight, histopathology) and thyroid hormone levels.

In a second step, the Panel considered the nanoscale scoring in the assessment of thyroid and parathyroid toxicity. One study with RoB 3 and NSC 1 supported that no effects were observed for these endpoints. Due to the lack of reliable studies NSC 1 or 2, comparison of the outcome of studies NSC 4 with that of NSC 1 and 2 was not feasible.

Based on the available data, the Panel concluded that it is likely that E 551 causes no adverse effect on these organs.

Immunotoxicity and hypersensitivity, allergenicity and food intolerance

In this WoE analysis for immunotoxicity, 12 studies of different durations (28 days up to 1 year) all scoring 1 or 2 for the internal validity (RoB) have been considered by the Panel; seven studies were conducted in rats and five in mice. Regarding the nanoscale considerations, there was no study with NSC score 1, two studies scored NSC 2, three studies scored NSC 3 and seven studies scored NSC 4. The Panel considered the data set sufficient to investigate potential immunotoxicity in relation to the exposure of the tested animals to aggregates in the nano-range size and suitable for large agglomerates.

The data set included the following parameters: spleen weight and histopathology (9 studies), thymus weight and histopathology (4 studies), lymph nodes weight and histopathology (6 studies), B- and T-cell proliferation (2 studies), and endpoints related to markers of inflammation and immune reactions (plasma cytokines, OVA tolerance model, IgG antibodies) (5 studies).

No difference was found in thymus, spleen, lymph nodes weight and histopathology. While some studies indicate the possibility of pro-inflammatory effects, others present conflicting findings or demonstrate no notable effects. Overall, there is low confidence in the adversity of the findings due to the inconsistency of the results, concerning changes in further functional and morphological apical endpoints.

In a second step, the Panel considered the nanoscale scoring in the assessment. One study with RoB 1/2 and NSC 2 investigated spleen histopathology, T, B and cytotoxic cells and plasma cytokines. One study RoB 1/2 and NSC 2investigated T, B, natural killer and cytotoxic cells and Ig-anti-OVA. The other immunotoxicity endpoints were investigated in studies with NSC 3 and 4. One study with RoB 3 and NSC 1 supported the no effects on immunotoxicity endpoints. The Panel did not identify differences in the outcome between studies with NSC 2 vs. 3 and 4.

Based on the available data, the Panel concluded that it is likely that E 551 has no adverse effects on the immune system.

Reproductive and developmental toxicity

In this WoE analysis for reproductive and developmental toxicity, the Panel assessed seven studies in rats of different durations (28 days up to 1 year) all scoring 1 or 2 for the internal validity (RoB). Regarding the nanoscale considerations, two studies scored NSC 3 and five studies scored NSC 4; none had NSC 1 or 2. The Panel considered the data set suitable to investigate potential reproductive toxicity in relation to the exposure of the tested animals to large agglomerates (NSC 4), and of agglomerates and aggregates in the nanosize range with uncertainty on the degree of agglomeration (NSC 3).

The data set included the following parameters: reproductive organ weights (6 studies) and histopathology (5 studies), prostate weight (3 studies) and histopathology (5 studies), sperm analysis (1 study), fertility in females (3 studies) and pre- and postnatal developmental effects (2 studies).

No evidence of adverse effects on reproductive and developmental toxicity-relevant endpoints was found.

In a second step, the Panel considered the nanoscale scoring in the assessment of reproductive or developmental toxicity. One study with RoB 3 and NSC 2 supported no effects on reproductive or developmental toxicity. Due to the lack of reliable studies with NSC 1 or 2, the comparison of the outcome of studies NSC 3 and 4 with that of NSC 1 and 2 was not possible.

Based on the available data, the Panel concluded that it is likely that E 551 has no adverse effects on reproductive and developmental toxicity.

Neurotoxicity

In this WoE analysis for neurotoxicity, the Panel assessed a total of seven studies of different durations (28 days up to 1 year) all scoring 1 or 2 for the internal validity (RoB); six studies were conducted in rats and one in mice. Regarding the nanoscale considerations, two studies scored NSC 3 and five studies scored NSC 4. The Panel considered the data set suitable to investigate potential neurotoxicity in relation to the exposure of the tested animals for large agglomerates, and of agglomerates and aggregates in the nano-size range with uncertainty on the degree of agglomeration (NSC 3).

The data set included the following parameters: brain weight (7 studies) and brain histopathology (5 studies).

No study reported any adverse effects for neurotoxicity-relevant endpoints.

In a second step, the Panel considered the nanoscale scoring in the assessment of neurotoxicity. One study with RoB 3 and NSC 1 supported no effects on neurotoxicity. Due to the lack of reliable studies with NSC 1 or 2, the comparison of the outcome of studies NSC 3 and 4 with that of NSC 1 and 2 was not possible.

Based on the available data, the Panel concluded that it is likely that E 551 has no neurotoxic effects.

Pancreas toxicity

In this WoE analysis for pancreas toxicity, the Panel assessed a total of four rat studies of different durations (28 days up to 1 year), all scoring 1 or 2 for the internal validity (RoB). Regarding the nanoscale considerations, all studies scored NSC 4. The Panel considered the data set suitable to investigate potential pancreas toxicity in relation to the exposure of the tested animals for large agglomerates (NSC 4).

The data set included the following parameter: pancreas histopathology (4 studies).

No study reported any adverse effects on pancreas.

In a second step, the Panel considered the nanoscale scoring in the assessment. Due to the lack of reliable studies with NSC 1 or 2, the comparison of the outcome of studies NSC 4 with that of NSC 1 and 2 was not possible.

Based on the available data, the Panel concluded that it is likely that E 551 has no adverse effects on pancreas toxicity.

Endogenous metabolism

In this WoE analysis for endogenous metabolism, the Panel assessed a total of eight studies of different durations (28 days up to 1 year) all scoring 1 or 2 for the internal validity (RoB). Five studies were conducted in rats and three in mice. Regarding the nanoscale considerations, three studies scored NSC 3, and five studies scored NSC 4. The Panel considered the data set suitable to investigate potential pancreas toxicity in relation to the exposure of the tested animals for large agglomerates (NSC 4), and of agglomerates and aggregates in the nano-size range with uncertainty on the degree of agglomeration (NSC 3).

The data set included the following parameters: cholesterol (total, HLD, LDL) and triglycerides (6 studies), glucose tolerance test (1 study), serum glucose (5 studies).

No study reported any adverse effects on endogenous metabolism.

In a second step, the Panel considered the nanoscale scoring in the assessment. Due to the lack of reliable studies with NSC 1 or 2, the comparison of the outcome of studies NSC 3 and 4 with that of NSC 1 and 2 was not possible.

Based on the available data, the Panel concluded that it is likely that E 551 has no adverse effects on endogenous metabolism parameters.

Gastrointestinal tract toxicity

In this WoE analysis for gastrointestinal toxicity, the Panel assessed a total of nine studies of different durations (28 days up to 1 year), all scoring 1 or 2 for the internal validity (RoB). Five studies were conducted in rats and four in mice. Regarding the nanoscale considerations, one study scored NSC 2, two studies scored NSC 3 and six studies scored NSC 4. The Panel considered the data set sufficient to assess potential gastrointestinal tract toxicity in relation to the exposure of the tested animals to aggregates in the nano-range size and suitable for the exposure to large agglomerates.

The data set included the following parameters: stomach histopathology (5 studies), small and large intestine histopathology (9 studies), small and large intestine weight (1 study).

No study reported any adverse effects on gastrointestinal tract.

In a second step, the Panel considered the nanoscale scoring in the assessment. This comprised one study with NSC 2 and eight studies with NSC 3 or 4. The Panel did not identify any differences in the outcome between studies with nanoscale score 2 vs. 3 and 4.

Based on the available data, the Panel concluded that it is likely that E 551 has no adverse effects on gastrointestinal tract toxicity based on the available data.

Lung toxicity

In this WoE analysis for lung toxicity, the Panel assessed a total of six studies of different durations (28 days up to 1 year) all scoring 1 or 2 for the internal validity (RoB). Four studies were conducted in rats and two in mice. Regarding the nanoscale considerations, one study scored NSC 2, one study scored NSC 3 and four studies scored NSC 4. The Panel considered the

data set sufficient to assess potential lung toxicity in relation to the exposure of the tested animals to aggregates in the nano-range size and suitable for the exposure to large agglomerates.

The data set included the following parameters: lung weight (2 studies) and lung histopathology (6 studies).

No study reported any adverse effects on lung.

In a second step, the Panel considered the nanoscale scoring in the assessment. This comprised one study with NSC 2 and five studies with NSC 3 or 4. One study with RoB 3 and NSC 2 supported no effects on lung. The Panel did not identify any differences in the outcome between studies with NSC 2 versus 3 and 4.

Based on the available data, the Panel concluded that it is likely that E 551 has no adverse effects on lung toxicity.

Bone toxicity

In this WoE analysis for bone toxicity, the Panel assessed four rat studies of different durations (28 days up to 1 year) all scoring 1 or 2 for the internal validity (RoB) have been considered by the Panel; all studies have NSC 4. The Panel considered the data set suitable to investigate potential bone toxicity in relation to the exposure of the tested animals for large agglomerates (NSC 4), but the aggregates in the nano-range size were not tested.

The data set included the following parameter: sternum histopathology, including bone marrow analysis (4 studies).

No study reported any adverse histopathology of the sternum.

In a second step, the Panel considered the nanoscale scoring in the assessment of bone toxicity. Due to the lack of reliable studies with NSC 1 or 2, comparison of the outcome of studies NSC 4 with that of NSC 1 and 2 was not feasible.

Based on the available data, the Panel concluded that it is likely that E 551 has no adverse effects on bone toxicity.

Vascular toxicity

In this WoE analysis for vascular toxicity, the Panel assessed a total of eight studies of different durations (28 days up to 1 year), all scoring 1 or 2 for the internal validity (RoB). Four studies were conducted in rats and four in mice. Regarding the nanoscale considerations, one study scored NSC 2, two studies scored NSC 3, and five studies scored NSC 4. The Panel considered the data set suitable to investigate potential bone toxicity in relation to the exposure of the tested animals for large agglomerates (NSC 4), but the aggregates in the nano-range size were not tested.

The data set included the following parameters: heart weight (5 studies), heart histopathology (6 studies) and aorta histopathology (4 studies).

No study reported any adverse effect on histopathology of the heart and aorta, and heart weight.

In a second step, the Panel considered the nanoscale scoring in the assessment of vascular toxicity. One study with RoB 3 and NSC 2 supported no effects on vascular toxicity. The Panel did not identify any differences in the outcome between studies with NSC 2 vs. 3 and 4.

Based on the available data, the Panel concluded that it is likely that E 551 has no adverse effects on vascular toxicity.

3.5.12 | Hazard characterisation and MOE

Hazard characterisation

The Panel considered that based on the WoE analysis of the studies all conducted with SAS relevant for the assessment of the food additive, E 551 did not elicit adverse health effects in experimental animals in the tested dose range up to 5000 mg/kg bw per day (the highest dose tested) administered via the oral route. Among the available toxicological studies, the NOAEL of the highest dose tested was considered suitable for the identification of a reference point if the study was performed in rats and included at least two dose groups and a control group. Within these studies, a 12-month study in rats (BSL Bioservice, 2021, documentation provided to EFSA No. 4) was considered preferable because of the study duration. However, in the case of E 551, nano-specific considerations have to be taken into consideration. This 1-year study was scored NSC 4, indicating that its results cannot be used to assess the safety of the portion of E 551 present as aggregates in the nano-range. In the available data set, there is no other study, of any duration, having the same level of internal validity (RoB 1 or 2), and higher NSC (1 or 2), that included at least two dose groups and a control group, which could be used for assessing the safety of E 551 when present as aggregates in the nano-range.

Among the available studies RoB1/2 and with NSC 3, the publication of van der Zande et al. (2014) reported two studies in rats: The first was a 28-day study with three doses and control, the highest dose being 2047 mg/kg bw per day. The second study had a duration of 84 days and only one dose of 2047 mg/kg bw per day was tested against control. The 28-day study fulfils the condition for the study selection with at least two dose groups and a control group. In addition, the 84-day study informs about longer exposure effects at a high dose. In this study with the dose of 2047 mg/kg bw per day, no adverse effects were observed. The identification of a reference point at the NOAEL in the 84-day study with a full range of measured endpoints, although performed with only one dose, is supported by the NOAEL of 2047 mg/kg bw per day in the 28-day which measured a limited number of endpoints, however performed with three doses and a control group. The Panel considered that the dose of 2047 mg/kg bw per day, the highest dose tested without adverse effects after 84 days of exposure, provides the relevant reference point. This study, despite being of shorter duration, takes better account of the

nanoparticle characteristics (NSC 3) than the 1-year study (NSC 4). The reference point is supported by the 1-year study in which the highest dose tested without adverse effects was 1000 mg/kg bw per day, taking into account that the half-life is 15 days and the steady state has therefore already been reached by 84 days (see Section 3.5.1).

Selection of assessment factors

For the derivation of an appropriate MOE, the Panel considered Si as a surrogate for SAS particles (see Section 3.5.1). The Panel further considered whether the conventional default adjustment factor of 100 would be appropriate in the case of E 551 or whether a chemical-specific adjustment factor approach would be more appropriate.

The default factor of 100 is composed of a factor of 10 to account for interspecies differences between test species and humans, and a second factor of 10 for interindividual differences in the human population. These two factors allow for interspecies differences and human variability and consider both toxicokinetics (TK) and toxicodynamics (TD). For the TK component of the interspecies factor, a value of 4 is used when the extrapolation is made from rat to human (EFSA Scientific Committee, 2012). This factor 4 is based on allometric scaling from rat to human. The other factor of 2.5 accounts for interspecies differences in TD. For interindividual differences, the factor 10 is subdivided into two factors of 3.2 each, to account for TK and TD differences. The default interindividual factor of 10 for variability in the human population appears justified based on analysis of variability of response to drug dosages, but the subdivision into two interindividual factors of 3.2 for TK and TD is not well supported by data.

In 2005, the WHO/IPCS proposed a framework indicating how chemical-specific TK and/or TD data can be used to replace the default factors or its subfactors (WHO/ICPS, 2005). In line with these suggestions, following the extended approach as described in the publication of Bhat et al. (2017), quantitative analysis of TK and TD can be used to develop chemical-specific MOE factors. In the absence of an adverse effect of SAS, no data are at hand to inform about chemical-specific interspecies and intraspecies TD factors, and hence the default factors have to be used i.e. 2.5 for the interspecies difference and 3.2 for intraspecies variability. The TD factor is thus overall, 8 (2.5×3.2).

However, for TK, information which can be used to derive chemical-specific factors is available. As described in the ADME section (see Section 3.5.1), SAS particles are taken up by endocytosis, leading to an internal/systemic exposure. Whereas two publications (Chu et al., 2011; Halamoda-Kenzaoui et al., 2017) deal with specifying the type of endocytosis of SiO_2 nanoparticles in the CaCo-2 cell and other cellular models (caveolae-mediated vs. non-caveolae-mediated), no information is available in the literature from in vivo studies in the small intestine of different species, including humans. Since the absorption of SiO_2 is an active energy-demanding process and that small rodents have a higher basic metabolism than humans, the Panel presumed conservatively that the absorption is qualitatively and quantitatively similar between species and within a species. After endocytotic uptake, E 551 is present in the cells of different organs where it is dissolved to silicic acid ($Si(OH)_4$) (see Section 3.5.1). In urine, Si is found mainly in the form of silicic acid, indicating that the systemically available SAS particles dissolve and that silicic acid is formed and excreted via glomerular filtration (Jurkić et al., 2013; Kwon et al., 2024). Comparing data of glomerular filtration rate (GFR) between rat and human revealed that the median value of GFR is 4.0 mL/min/kg bw in rats, 1.6 mL/min/kg bw in healthy adults and 0.9 mL/min/kg bw in the elderly (> 65 years of age) (Smeraldi et al., 2020). Multiplying the ratio of the GFR distributions of the rat GFR/healthy human GRF with the ratio of the GFR distributions of the healthy humans GFR/elderly humans GRF yields a factor of 4.5 (3.3–6.7) which is accounting for both the interspecies (rat to human) and the intraspecies (healthy human to elderly) TK factor (Smeraldi et al., 2020). From the excretion, a TK factor is 4.5.

The overall specific assessment factor, appropriate for E 551, is thus $8 \times 4.5 = 36$ for studies in rat. Therefore, the Panel considered that an overall assessment factor of 36 should be applied for the assessment of the MOE. This means that the MOE, i.e. the ratio between the reference point and the estimated exposure, should be at least 36 for not raising a safety concern.

Calculation of the MOE and risk assessment

In its 2018 opinion, the ANS Panel had considered that it would have been possible to derive an ADI for the food additive E 551 should the limitations in the toxicological database be reduced. Hence, in the absence of genotoxicity concern, the FAF Panel considered which approach to assess the safety of E 551 would be appropriate taking into account that no adverse effect was seen in all animal studies considered in the present assessment up to a dose of 5000 mg/kg bw per day. It has also to be taken into consideration that uncertainty exists to which extent small aggregates in the nanosize range were tested in the animal studies. Nanoscale score 1 or 2 could not be assigned to any of the studies, i.e. the studies were not performed with a fully dispersed material consistent with the EFSA Guidance on Nano – Risk Assessment (EFSA Scientific Committee, 2021b). Other studies were scored NSC 4 which indicates that, in those studies, large agglomerates were tested. This applies particularly to the 12-month study (BSL Bioservice, 2021, documentation provided to EFSA No. 4) and the studies investigating reproductive and developmental endpoints; therefore, these studies cannot inform with certainty about the potential toxicity of the nanosize aggregates.

Given the remaining uncertainties resulting from the limitations of the available database, the FAF Panel considered that it is not appropriate to derive an ADI. The Panel considered that the MOE approach should be applied to evaluate the safety of E 551.

For identifying a reference point the Panel considered that, from the available toxicity data set, the highest dose tested in the chronic study in rats (BSL Bioservice, 2021) was 1000 mg/kg bw per day and the highest dose tested in the 84-day study in rats (van der Zande, 2014) was 2047 mg/kg bw per day.

The Panel considered that the dose of 2047 mg/kg bw per day, the highest dose tested without adverse effects after 84 days of exposure, provides the relevant reference point. This study, despite being of shorter duration, takes better account of the nanoparticle characteristics (NSC 3) than the 1-year study (NSC 4). The reference point is supported by the 1-year study in which the highest dose tested without adverse effects was 1000 mg/kg bw per day, taking into account that the half-life is 15 days and the steady state has therefore already been reached by 84 days.

According to the exposure assessment in the EFSA 2018 opinion (EFSA ANS Panel, 2018), the ANS Panel discussed the various exposure scenarios and considered that the refined 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 FAF Panel followed the consideration of the ANS Panel, and used the non-brand loyal exposure levels for the calculation of the MOE (Table 10).

TABLE 10 Ranges of MOEs based on the dietary exposure estimates considering the refined non-brand-loyal exposure scenario, in six population groups (mean and 95th Percentile). Reference point 2047 mg/kg bw per day (84-day study by van der Zande et al., 2014).

	Infants (12 weeks to 11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Mean (lower – upper level)	2559–386	682–277	758–111	1204–499	2274–758	2924–787
95th percentile (lower – upper level)	602–151	266–137	366–41	525–230	890–320	1204–366

Taking the reference point of 2047 mg/kg bw per day, the Panel noted that the MOE in all population groups was above 36, indicating that there would be no safety concern at the reported use and use levels.

3.5.13 | Considerations on the margin of internal exposure

The Panel elaborated the following considerations on the internal exposure to support the calculation of the MOE and the conclusion of no safety concern for E 551.

In risk assessment, the doses of the substance in question are normally given in external doses; i.e. the estimated consumer exposure and the doses applied in animal testing are external doses. However, it is the internal dose which is eliciting the effect and is therefore the relevant metric. This consideration applies for all substances. However, there is an important difference between particulate nano-sized material and non-particulate material. For the non-particulate material, it can generally be assumed that the relationship between external dose and internal dose is linear. This means that, with increasing external dose, the internal dose will increase proportionally. For particulate nano-sized material, agglomeration may increase with increasing external dosing concentrations. Because agglomeration will likely reduce the fraction absorbed, the internal exposure after external oral exposure does not increase proportionally with dose. In the case of E 551, the internal dose was estimated from experimental data. The results indeed showed that the internal dose did not proportionally increase with increasing external dose (see Section 3.5.1).

To complement the risk assessment based on external doses, the Panel used existing data to compare the mean Si concentration in organs in humans to the highest Si concentration in the same organs in animal toxicity studies. The toxicokinetic studies showed that liver and spleen are the organs in which the highest Si concentrations are found. For subchronic studies, e.g. 90-day oral toxicity studies, steady state in tissue concentrations is reached. Data on the Si content in liver and spleen in rodents are taken from the literature assessed in this evaluation (see ADME section). No adverse effects were observed in these animal studies.

Data on the Si concentration in human liver and spleen are reported by Peters et al. (2020). The authors investigated the Si concentration in liver and spleen of 15 deceased persons (age of 64–98 years). It can be assumed that these persons were exposed to silicon from all sources, and that Si concentrations per individual do not deviate from the steady-state conditions.

Because in both humans and animals, tissue levels are likely to be at the steady state, it is justified to compare Si concentrations in the liver and spleen reported in humans with the highest tissue Si concentrations in animal subchronic studies (see Section 3.5.1).

Liver Si concentrations are reported as 75 mg/kg in mice (Lu et al., 2021, ref 5985), and in rats as 90 mg/kg (Lee et al., 2017, ref 1452), 55 mg/kg (Yoo et al., 2021, ref 3367) and 110 mg/kg (van der Zande et al., 2014, ref 13,978). In humans, the liver Si concentration showed an average value of 8 ± 8 mg Si/kg liver ($n = 15$) (Peters et al., 2020). From the analysis of the animal data, it can be deduced that the liver Si concentration without toxicologically relevant effects is approximately up to 100 mg Si/kg liver. Comparing the average liver Si concentration in humans of 8 mg Si/kg liver with the liver Si concentration without effects of 100 mg/kg, the margin of internal exposure is 12.5.

Spleen Si concentrations are reported as 100 mg/kg (Lu et al., 2021) in mice and 116 mg/kg (Van der Zande et al., 2014) in rats. In humans, the spleen Si concentration showed an average value of 12 ± 12 mg Si/kg spleen (n = 15) (Peters et al., 2020). From the analysis of the animal data, it can be deduced that the spleen Si concentration without toxicological relevant

effects is up to 110 mg Si/kg spleen. Comparing the average spleen Si concentration in humans of 12 mg Si/kg spleen with the spleen Si concentration in experimental animals without effect of 110 mg/kg, the margin of internal exposure is 10.

For the MOE calculation, the default value for the toxicodynamic interspecies difference and the intraspecies variability is 8 (2.5 × 3.2), because inter- and intra-species toxicokinetic differences do not have to be considered when comparing internal concentrations, and only toxicodynamic differences are relevant.

The Panel considered that this additional analysis supports the conclusion of no safety concern for E 551 at the reported use and use levels for the general population.

3.6 | Assessment of E 551 as food additive in formulae for infants below 16 weeks of age

The following was requested in the EFSA call for data[2]:

Within the frame of the EFSA Guidance of the Scientific Committee on the risk assessment of substances present in food intended for infants below 16 weeks of age the following information on the toxicological properties of silicon dioxide (E 551) is required:

- Performance of an Extended One Generation Reproductive Toxicity Study (EOGRTS) in accordance with OECD TG 443 (OECD, 2018a). Post-marketing surveillance reports on undesired and adverse reactions, indicating the ages and other relevant data of the exposed infants and young children and the use levels in the marketed products.

According to Annex III, Part 5, Section B of Regulation (EC) No 1333/2008,[50] silicon dioxide (E 551) is authorised as a food additive to be added in nutrients intended to be used in foods for infants and young children. To conclude on the safety of this use of E 551 in food for infants below 16 weeks of age (food categories (FC) 13.1.1 'Infant formula' and 13.1.5.1 'Dietary foods for special medical purposes and special formulae for infants'), the FAF Panel assessed the information provided by the IBO. Dietary exposure to E 551 from its use as a food additive in foods for infants below 16 weeks of age was estimated (see Section 3.3).

The Panel considered that the submitted toxicological data could not be used for the assessment of the safety of E 551 for its uses in food for infants below 16 weeks of age. The two-generation reproduction toxicity study without direct dosing of the pups (TNO Triskelion, 2012, documentation provided to EFSA No. 18) described in full in section 3.3.6, did not provide adequate exposure to the pups. Exposure through dam's milk in experimental studies is usually substantially lower than the exposure through feed and the resulting doses in the neonatal animals are considered insufficient for hazard characterisation (EFSA Scientific Committee, 2017). This was supported by the fact that the presence of SiO_2 particles could not be demonstrated in the analysed samples of the milk of rats (see Section 3.2). Hence, the Panel could not use this study to conclude on the safety of E 551, in food for infants below 16 weeks of age. A study in neonatal animals recommended in the EFSA Scientific Committee Guidance (EFSA Scientific Committee, 2017) was not available.

The Panel took an alternative approach using data on adverse events reported in the regulatory database EudraVigilance[51] for the medicine simeticone. Simeticone, which is sold on the market, contains between 4% and 7.5% silica as the active ingredient. The monograph for simeticone in the European Pharmacopoeia indicates a content of 4%–7% silica in this medicine (European Pharmacopoeia 11, 2023).

An IBO has indicated that the SiO_2 which is used in one brand of simeticone[52] is SAS used as E 551 (Documentation provided to EFSA No. 30).

Simeticone as a medicine has been on the market for decades (Danielsson & Hwang, 1985). Simeticone is used to alleviate meteorism and flatulence, specifically in infants and young children. For infants who are bottle-fed the dosing advice is to add it directly into the bottle (see the Summary of Product Characteristics (SPC), information for professionals).

In the EudraVigilance database, 218 events associated with simeticone are documented for the age group 0–2 years (EMA communication to EFSA). The adverse events which are most frequently reported involve erythema, rash, constipation, vomiting, diarrhoea, crying, somnolence, urticaria, abdominal pain, flatulence, pain, pruritus. The Panel considered that none of the reported adverse events could be attributed to silica, based on their unspecific character and in consideration of the properties of simeticone.

The Panel further noted that no side effects were reported in the patient information leaflet (PIL) and the Summary of Product Characteristics (SPC), both of which represent official regulatory documents subject to scrutiny by the regulatory authorities.

Given this information, the Panel noted that from the medicinal use of simeticone, containing up to 7.5% silica, there is no indication that SiO_2 can elicit adverse effects in infants below 16 weeks of age.

The exposure to silica from the use of simeticone medicine can be calculated using dosing information provided in PIL and in the SPC across various age groups. Age specific body weights can be taken from EFSA Guidance on the risk

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

[51]EudraVigilance is the system for managing and analysing information on suspected adverse reactions to medicines which have been authorised or studies in clinical trials in European Economic Area (EEA). The European Medicines Agency (EMA) operates the system in behalf of the European Union (EU) medicines regulatory network. The EudraVigilance system is working since December 2001.

[52]SILFAR-S-184-en-2022.07.08.pdf(wacker.com).

assessment of substances present in food intended for infants below 16 weeks of age (EFSA Scientific Committee, 2017). According to the WHO child growth standards,[53] a 1-month-old infant typically weights 3.5 kg. Therefore, the SiO_2 exposure from the use of simeticone, containing 7.5% SiO_2 would amount to 2.37 mg/kg bw per day for 1-month-old infants, 1.91 mg/kg bw per day for 2-month-old infants, 1.55 mg/kg bw per day for 3-month-old infants and 1.36 mg/kg bw per day for 4-month-old infants. This exposure range is comparable to the estimated dietary exposure for the use of food additive E 551 in food for infants below 16 weeks of age (see Table 6).

The Panel considered that the data on adverse events reported in the EudraVigilance database, and the assessment performed by the EU Member States competent authorities concerning medicinal products containing simeticone, give no indication of relevant adverse health effects from silica (active ingredient), up to an exposure of 2.37 mg/kg bw daily in infants, an exposure similar to the exposure with SAS as a food additive in food for infants below 16 weeks of age.

In summary, no specific studies were available in which the safety of E 551 was adequately investigated in very young animals or in infants and young children. However, no adverse effects were seen in animal studies at the highest dose tested (5000 mg/kg bw per day). Considering information from Eudra-Vigilance database for the age group 0–2 years and the information in the SPCs, the Panel considered that there is no safety concern of SAS as an active ingredient of simeticone. Overall, the Panel concluded that there is no safety concern for the use of E 551 as food additive according to Annex III to Regulation (EC) no 1333/2008 in food intended for infants below 16 weeks of age at the current exposure levels.

3.7 | Uncertainty analysis

Due to the large amount of information reviewed in the limited time available and new data submitted by IBOs only recently, a structured uncertainty analysis in line with the EFSA Guidance on Uncertainty Analysis in Scientific Assessments (EFSA Scientific Committee, 2018) was not possible. Instead, the Panel identified and considered the following uncertainties during the conclusion stage of the risk assessment of E 551. Despite the available data, there is uncertainty on the size/morphology of the aggregates for the different SAS used as E 551. Furthermore, no information on the relative amounts of particles occurring as isolated particles, aggregates and/or as agglomerates in food is available, and in addition, no quantitative information on the level of aggregation/agglomeration of E 551 in food is known.

The data submitted by the IBOs in response to the follow-up call issued to address the uncertainties identified by the ANS Panel on the characterisation of SiO_2 used as a food additive has led to the identification of around 70 different products commercialised as E 551. Since the size and morphology of the aggregates could have an impact on the cellular uptake and toxicokinetics, and therefore, the potential toxicity, such variation in the aggregates of SAS (size and morphology) between the different types of SAS may introduce an uncertainty on the extrapolation of the results of studies performed with a specific SAS to all materials used as E 551. Different types of SAS were tested in different systems and experimental conditions; because all the included toxicological studies have been conducted with SAS compliant with the definition of E 551 (as described in Section 3.4.3), the Panel considered that the available data set was sufficient to cover all the main toxicity endpoints; nonetheless, it has to be remarked that not all SAS materials used as E 551 have been tested for all toxicity endpoints including, genotoxicity.

The paucity of toxicological studies other than genotoxicity studies with proper dispersion protocol, i.e. with NSC 1 or 2, creates uncertainty in the present assessment of the potential toxicological effects related to the exposure to E 551 nano-size aggregates. This consideration does not apply to the genotoxicity studies as most of the in vitro and in vivo tests have NSC 1 or 2; the Panel considered that the conclusion on the genotoxicity is applicable to nano aggregates present in E551.

With respect to the biological and toxicological data, systemic availability of SiO_2 cannot be directly estimated through the quantification and measurements of the particles but rather relies on the measurement of Si as a proxy. The natural and varying background levels of Si resulting from its occurrence in the diet of the experimental animals, including controls, represent an important source of uncertainty in the interpretation of the data on systemic availability. Furthermore, because information on the Si concentrations was not available for all organs and tissues, the systemic exposure is, in principle, underestimated. Nonetheless, some publications report negligible Si concentration in other organs not included in the estimation of systemic availability.

Quantitative information on the absorption of E 551 by humans from food and drinks is restricted to extrapolation from data in rodents, in which there is some uncertainty about the degree of agglomeration of E 551 in the administration matrix. This uncertainty particularly concerns the degree to which agglomeration and gel formation of the material occurs, especially at high doses. Studies often lack or provide only limited information on the degree of agglomeration of SAS suspensions tested.

For the risk assessment of E 551 when used in foods intended for use in infants below 16 weeks of age, uncertainty exists as to whether a specific SAS, also used as E 551, which is one of the ingredients in a medicinal product of simeticone, is fully representative of other SAS used in other simeticone medicinal products. No adverse reactions related to SiO_2 used in the drug simeticone, used for decades, are mentioned in the EudraVigilance database, in patient leaflets and/or SPCs. In this light, the uncertainty related to the limited representativeness of the specific SAS in simeticone medicinal products is considered not to have an impact on the final assessment.

[53] https://www.who.int/tools/child-growth-standards/standards.

There is uncertainty on the ability of SAS to modify the intestinal immune homeostasis by inducing low-grade intestinal inflammation, which might predispose to food allergy and autoimmune-mediated celiac diseases. This uncertainty is related to the fact that there are only two studies available for assessment, reporting inconsistent apical effects.

In the reproductive and developmental studies (see Section 3.5.6) and 12-month study (BSL Bioservice, 2021, documentation provided to EFSA No. 4) (see Section 3.5.5), which were performed according to the OECD guidelines, no adverse effect was observed up to the highest dose tested (1000 mg/kg bw per day). In accordance which these guidelines, no histopathological investigation was done on low- and mid-dose groups in the absence of effects in the high-dose group. The Panel noted, considering the nano-specific aspects of E 551 and possible effects at lower doses, that the analysis of the low- and mid-dose groups could have been helpful.

4 | DISCUSSION

Based on the available data submitted for different commercialised E 551, the food additive can be described as synthetic amorphous silica (SAS), without crystalline structure;

- Different types of SAS are included:
  - Produced by the pyrolysis method: fumed (or pyrogenic) silica,
  - Produced by the wet method: precipitated silica, silica gel and hydrous silica;
- SAS consists of near-spherical nano-sized constituent particles (with the majority exhibiting D50 from 2 to 28 nm) forming complex (fractal-like) aggregates (this means that non-aggregated colloidal silica, also referred as Stöber silica, is not used as E 551);
- SAS is not coated or surface functionalised.

Analytical data on lead, mercury, arsenic and aluminium in samples of different types of SAS used as E 551 were submitted to EFSA (Section 3.4.1). Results of the analysis after using different sample preparation methods (i.e. partial digestion (with HNO_3), full digestion with HF and 0.5 M HCl extraction) were available. The FAF Panel considered that the analysis of impurities after HCl extraction better reflects the bioavailable content of the toxic elements and is a method sufficiently conservative to assess the exposure to those impurities from the use of E 551. Considering the results of the analysis by HCl extraction, concentration data were reported for lead in 11 out of the 17 analysed samples, up to 2.1 mg/kg (LOQ 0.1 mg/kg). Mercury and arsenic were reported below the LOQ, 0.05 and 0.1 mg/kg, respectively. The content of aluminium in different E 551 ranged from below 1 to 420 mg/kg. The Panel noted that the occurrence data submitted for mercury and arsenic in E 551 are substantially lower than the current limits in the EU specifications.

The Panel performed the risk assessment that would result if the toxic elements were present in E 551 at: (i) the existing maximum limit in EU specifications; (ii) at the levels from the analytical data reported with the HCl extraction method. For this second scenario, the rounded up highest measured value of the level of the toxic element was used, if quantified (this was only possible for lead 2.5 mg/kg and aluminium 450 mg/kg). In the absence of any measured value(s), the reported LOQ was used, after applying a factor of 5 to allow flexibility with respect to representativeness and homogeneity (i.e. resulting in 0.5 mg/kg for arsenic and 0.25 mg/kg for mercury). The resulting figures in Table E.4 (Appendix E) show that for lead and mercury, the presence of these toxic elements in E 551 either at the current specifications limit values or at the levels selected by the Panel would not give rise to concern, this also applies to aluminium, considering the rounded up highest measured value. For arsenic, taking into account the use of E 551 for the general population, the calculated MOE values, considering the current limit in the EU specifications, were considered to be insufficient.

Based on the calculations performed by the Panel (Table E.4) and the fact that the food additive is not the only potential dietary source of toxic elements, the Panel recommends to lower the maximum limits for lead, mercury and arsenic and to introduce a maximum limit for aluminium.

Dietary exposure to silicon dioxide (E 551) for the general population was estimated at the time of the re-evaluation in 2017 (EFSA ANS Panel, 2018). Considering that no new occurrence data are available to EFSA since 2018, and that dietary surveys from the EFSA comprehensive database giving the highest exposure estimates in the 2017 re-evaluation are still in the consumption database, no new exposure estimation was performed for the general population.

The Panel considered appropriate to refer to the dietary exposure assessment performed by the ANS Panel from the 2018 opinion, and reconfirmed the choice of the refined, non-brand loyal scenario as the most appropriate and realistic scenario for risk characterisation of E 551 for the general population. In the ANS Panel opinion, the safety of E 551 for infants below 16 weeks of age was not addressed; therefore, the FAF Panel was requested to assess the safety of the use of E 551 in food intended for this population group.

Exposure to E 551 from its use as a food additive in formulae and formulae for special medical purpose intended for infants below 16 weeks was estimated based on the recommended infant formula consumption levels from SC Guidance (EFSA Scientific Committee, 2017). The maximum permitted level of E 551 refers to nutrient preparations intended to be added to infant formula. However, the resulting levels of E 551 in the final food are not known. Therefore, a regulatory maximum level exposure assessment scenario cannot be estimated. Levels of E 551 in both formulae consumed by infant

below 16 weeks of age (FCs 13.1.1 and FC 13.1.5.1) were provided by an IBO. The maximum and the average of all levels for each food category were used for estimating the exposure to E 551. For 13.1.1 and 13.1.5.1, for the scenario using the maximum level reported by the IBO, the dietary exposure was estimated to be 2 and 3 mg/kg bw per day (mean and high consumption, respectively). For FC 13.1.1, for the scenario using the mean levels reported by industry, the exposure considering mean and high consumption was calculated as 0.38 mg/kg bw per day and 1.1 mg/kg bw per day, respectively. For FC 13.1.5.1, the exposure considering the mean and high consumption was calculated as 0.6 mg/kg bw per day and 0.8 mg/kg bw per day, respectively. There is uncertainty on the representativeness of the levels reported by industry which were used in the calculations. This gives a potential for possible over- and under-estimation of exposure. It is noted that food under FC 13.1.5.1 could be consumed also by infants above 16 weeks of age and young children (or toddlers). In the current opinion, the dietary exposure for this food category was calculated only for infants below 16 weeks of age. It is noted that, during the re-evaluation, the ANS Panel did not perform an exposure assessment for the food for special medical purpose (FSMP) consumer only scenario (consumers of food under FC 13.1.5.1) since 'only one-use level was reported by industry on a niche product of FSMP described as special infant formulae'.

The toxicokinetics of E551 have been investigated in vitro and in vivo studies. In the in vitro studies, endocytotic uptake mechanism of SAS in cells and its intracellular distribution were demonstrated qualitatively. Qualitative data on particles localised in several organs were available only in a small number of in vivo studies. The kinetic information is based on measurements of Si in tissues at different time points. Because of high background levels due to the content of Si in feed, the low concentrations to be measured and technical challenges in the quantification, uncertainty exists on the results. While acknowledging the uncertainties, the Panel nonetheless used the results to estimate half-life and accumulation over time and systemic availability of SiO_2. Very low systemic availability is expected, but until now has not been adequately quantified (see Section 3.5.1). The data also showed that the relative systemic availability, when expressed as a percentage of the dose, decreased with increasing dose, probably because of a saturated uptake process.

After endocytotic uptake, E 551 is present in the cells of different organs where it is dissolved to silicic acid ($Si(OH)_4$) (see Section 3.5.1). Silicic acid is partly dissociated and silicic acid and Si in ionic form are excreted by the kidneys by glomerular filtration.

The reliability and relevance of the genotoxicity studies have been assessed by the EFSA ccWG genotoxicity based on their approach (EFSA, 2023). Based on the currently available data set, the Panel concluded that E 551 does not raise a concern for genotoxicity. Regarding the induction of DNA damage in spleen in one study after oral exposure of rats to NM-203 for 90 days at doses up to 50 mg/kg bw per day (Villani et al., 2022), the Panel took into account the high intra-group variability of the effect observed in spleen after oral treatment for 90 days and the lack of historical control data for this study, and considered that, in another study (Guichard et al., 2015), no induction of DNA damage was observed in spleen after intravenous injection which resulted in higher exposure of the spleen than after oral treatment. Therefore, the Panel considered that the result in the Villani et al. (2022) study does not change the overall conclusion on genotoxicity.

Regarding the nanoscale considerations, the appraisal step led to the grading of the studies into four categories, ranging from 1 to 4, from the highest level of confidence that nano-sized particles/aggregates were tested under the experimental conditions (NSC score 1), to the lowest (NSC score 4).

Regarding NSC, considering that the majority of in vitro and in vivo genotoxicity studies included in the present assessment have scores 1 or 2, the pattern of positive and negative results does not change significantly when considering the results all together (NSC 1–4) and separately for NSC 1–2. Therefore, the Panel considered that the conclusion on the genotoxicity is applicable to nano aggregates present in E551.

The in vivo toxicity studies other than genotoxicity have been assessed for their internal validity based on the OHAT Risk of Bias (RoB) Rating Tool for Human and Animal Studies (see Section 2.2), which has been modified and used for the appraisal of both animal and human studies. Only studies with internal validity (RoB) score 1 or 2 (RoB 1/2) were included in the further analysis of the toxicity. In selected cases, studies with RoB score 3 were used as supporting evidence of findings of studies with RoB score 1 and 2. Studies with RoB score 4 were not used in the assessment. The assessment of the overall body of evidence was conducted qualitatively by expert judgement.

Apart from the genotoxicity studies, among the in vivo toxicity studies with high internal validity (RoB1/2), there were none with NSC 1; two with NSC 2, while the majority of the studies were with NSC 3 or 4.

In the weight of evidence analysis, the Panel considered the data set of in vivo studies suitable to assess toxicological potential of large agglomerates (NSC 4), and of agglomerates and aggregates in the nanosize range, with uncertainty on the degree of agglomeration (NSC 3). Only two studies with NSC 2 (addressing general toxicity endpoints, liver, kidney, adrenals, immune, GI tract, vascular toxicity) using low doses and of short duration were available to assess the hazard of nanosize aggregates.

For general toxicity, no study reported adverse effects on general toxicity-relevant endpoints (body weight, appearance and behaviour, clinical signs, haematology, functional observations, mortality, blood electrolytes (Na, K, Mg, Cl, iP)) up to the highest dose tested of 5000 mg SAS/kg bw per day. Based on the WoE, the Panel considered it likely that E 551 has no adverse effects on general toxicity relevant endpoints; however, the considered studies can inform on the potential toxicity of nanosize aggregates/agglomerates only to a limited extent.

Liver and spleen are the two organs with the highest Si concentrations and content after administration of SAS. For both organs, no adverse effects were noted concerning the investigated parameters (weight, histopathology and liver enzymes) up to the highest dose tested of 5000 mg SAS/kg bw per day. Based on the WoE, the Panel considered it likely that E 551

has no adverse effects on liver and spleen; however, the considered studies can inform on the potential toxicity of nanosize aggregates/agglomerates only to a limited extent.

Furthermore, no adverse effects were found on other endpoints including weight and histopathology of the kidney, creatinine and urea serum levels, urinary tract histopathology up to the highest dose tested of 5000 mg/kg bw per day. No adverse effects were found in gastrointestinal tract, lungs, bones, endogenous metabolism parameters at oral doses up to or exceeding 1000 mg SAS/kg bw per day. Based on the WoE, the Panel considered it likely that E 551 has no adverse effects on the above-mentioned parameters. However, the considered studies can inform on the potential toxicity of nanosize aggregates/agglomerates only to a limited extent.

No adverse effects were found on adrenals weight or histopathology, thyroid/parathyroids or pituitary weight and histopathology, or on thyroid hormones at oral doses up to or exceeding 1000 mg SAS/kg bw per day. All studies had NSC 4. Based on the WoE, the Panel considered it likely that E 551 has no adverse effects on these organs and hormones. However, the considered studies cannot inform about the potential toxicity of nanosize aggregates/agglomerates.

In the weight of evidence analysis for reproductive and developmental toxicity, no adverse effects were reported at oral doses up to or exceeding 1000 mg SAS/kg bw per day. No adverse effects were reported at oral doses up or exceeding 1000 mg SAS/kg bw per day in neurotoxicity-relevant endpoints (brain weight and histopathology). Functional neurotoxicity endpoints (behaviour) were investigated in only one study, which due to high risk of bias (RoB 3), was not taken into account in the WoE. Based on the WoE, the Panel considered it likely that E 551 has no adverse effects on reproduction and development and no adverse effects on the nervous system. However, the considered studies addressing reproductive and developmental and neurotoxicity endpoints have NSC 3 or 4; therefore, they can inform on the potential toxicity of nanosize aggregates/agglomerates only to a limited extent.

Twelve publications with RoB 1/2 were reviewed to assess the immunotoxic potential of SAS. Various parameters related to immune system status were investigated across the studies, including blood leucocytes, lymphocyte subpopulations, cytokine production and immune-mediated disorders. Regarding the nanoscale considerations, there was no study with NSC 1. Two studies scored NSC 2, three scored NSC 3 and seven studies scored NSC 4. The considered studies can inform on the potential toxicity of nanosize aggregates/agglomerates only to a limited extent.

Pro-inflammatory effects were observed at certain doses, but without clear signs of organ damage. Additionally, SAS exposure provided indications of modifying intestinal immune homeostasis in a subchronic mouse study.

Studies conducted in male mice suggested a possible pro-inflammatory effect, while those in rats yielded inconsistent results, with some studies showing no effects and others reporting sex-dependent effects on immunological parameters. However, the ability of SAS to modify intestinal immune homeostasis by inducing low-grade intestinal inflammation, which might predispose to food allergy and autoimmune-mediated coeliac diseases, deserves further investigation, particularly in humans.

Due to the limited number of studies, differing doses and inconsistent findings, the Panel considered that although some findings might suggest immunotoxicity, there were apical effects not consistently reported. Based on the currently available data, the Panel concluded that it is likely that E 551 has no adverse effects on the immune system.

In its 2018 opinion, the ANS Panel considered that it would be possible to derive an ADI should the limitations in the toxicological data set be reduced. Hence, in the absence of a genotoxicity concern, the FAF Panel considered which approach to assess the safety of E 551 would be appropriate taking into account that no adverse effect was seen in all included animal studies up to a dose of 5000 mg/kg bw per day. It has also to be taken into consideration that uncertainty exists to which extent small aggregates in the nanosize range were tested in the animal studies. Nanoscale score 1 or 2 could not be assigned to any of the studies, i.e. the studies were not performed with a fully dispersed material consistent with the EFSA Guidance on Nano – Risk Assessment (EFSA Scientific Committee, 2021b). Most of the studies were scored nano score 4 which indicates that, in those studies, large agglomerates were tested. This applies particularly to the 1-year study and the studies investigating reproductive and developmental endpoints; therefore, these studies cannot inform with certainty about the potential toxicity of the nanosize aggregates.

Given the uncertainties resulting from the remaining limitations of the database, the FAF Panel considered that it is not appropriate to derive an ADI. The Panel considered that the MOE approach should be applied to evaluate the safety of E 551.

For identifying a reference point, the Panel considered that, in the included studies, the highest dose tested in the chronic study in rats (BSL Bioservice, 2021, Documentation provided to EFSA No. 4) was 1000 mg/kg bw per day and the highest dose tested in the 84-day study in rats (van der Zande et al., 2014) was 2047 mg/kg bw per day.

The Panel considered that the dose of 2047 mg/kg bw per day, the highest dose tested without adverse effects after 84 days of exposure, provides the relevant reference point. This study, despite being of shorter duration, takes better account of the nanoparticle characteristics (NSC 3) than the 1-year study (BSL Bioservice, 2021, NSC 4). The reference point is supported by the 1-year study in which the highest dose tested without adverse effects was 1000 mg/kg bw per day, taking into account that the half-life is 15 days and the steady state has therefore already been reached by 84 days.

Specific toxicokinetic considerations were applied allowing a reduction of the required MOE to 36 instead of the default value of 100 (see Section 3.5.12).

With regard to general population groups (above 16 weeks of age), taking the reference point of 2047 mg/kg bw per day, and the exposure calculated in the refined non-brand-loyal scenario, the Panel noted that the MOE was above 36, indicating that there would be no safety concern at the reported use and use levels.

To complement the risk assessment based on external doses, the Panel used existing data to compare the mean Si concentration in organs in humans to the highest Si concentration in the same organs in animal toxicity studies. Comparing

the average liver Si concentration in humans of 8 mg Si/kg liver with the liver Si concentration without effects of 100 mg/kg, the margin of internal exposure is 12.5 (see Section 3.5.13). Comparing the average spleen Si concentration in humans of 12 mg Si/kg spleen with the spleen Si concentration in experimental animals without effect of 110 mg/kg, the margin of internal exposure is 10.

The Panel considered that this additional analysis supports the conclusion of no safety concern for E 551 at the reported use and use levels for the general population.

The Panel was requested to assess the safety of E 551 in food for infants below 16 weeks of age. The exposure to E 551 in this age group was estimated at the maximum use levels as 3 mg/kg bw per day for the high consumption in both FC 13.1.1 and 13.1.5.1. The available hazard data set did not include any human or animal data on this age group. In the absence of this information, the Panel selected a particular approach in which the reported adverse events potentially related to simeticone, a medicinal product containing SAS, widely used for more than 40 years in infants, were considered. Information on adverse events were found in the EudraVigilance database of EMA. In addition, the information on side effects in the regulatory documents (patient leaflet, SPC) were scrutinised. Based on the information from Eudra-Vigilance database for this age group and the information in the SPCs, the Panel considered that there is no safety concern of SAS as an active ingredient of simeticone up to a dose of 2.37 mg/kg bw per day, the therapeutic dose in the relevant age group. The maximum highest exposure estimates (3 mg/kg bw per day) were comparable to the level without adverse side effects seen for simeticone. In addition, taking into account that no adverse effects were seen in animal studies at doses up to 5000 mg/kg bw per day, the Panel considered that there is no safety concern for the use of E 551 as food additive according to Annex III to Regulation (EC) no 1333/2008 in food intended for infants below 16 weeks of age at the current exposure levels.

5 | CONCLUSIONS

The Panel has addressed the data gaps specified in the recommendations made in the earlier scientific opinion of the ANS Panel on the re-evaluation of the safety of silicon dioxide (E 551) and has evaluated the safety of this food additive with respect to its permitted uses in foods for infants below 16 weeks of age. Based on the newly available information on the characterisation of the SAS used as E 551, and following the principles of the 2021 EFSA Guidance on Particle-TR, the conventional safety assessment has been complemented with nano-specific considerations. Given the uncertainties resulting from the remaining limitations of the database, the FAF Panel considered that it is not appropriate to derive an ADI, but to apply the MOE approach for the risk assessment. The Panel concluded that the MOE should be at least 36. The calculated MOEs considering the dietary exposure estimates for all population groups using the refined non-brand loyal scenario, estimated by the ANS Panel at the time of the 2018 re-evaluation, were all above 36.

The Panel concluded that E 551 does not raise a safety concern in all population groups at the reported uses and use levels.

The use of silicon dioxide (E 551) in food for infants below 16 weeks of age in FC 13.1.1 and FC 13.1.5.1 does not raise a safety concern at the current exposure levels.

The Panel also concluded that the technical data provided support an amendment of the specifications for silicon dioxide (E 551) laid down in Commission Regulation (EU) No 231/2012, as presented by the recommendations made in Table 8.

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ABBREVIATIONS

ADI	Accepted Daily Intake
ADME	Absorption, Distribution, Metabolism and Excretion
ALS	Angular Light Scattering

ALT	Alanine Aminotransferase
ANS Panel	EFSA Panel on Food Additives and Nutrient sources added to Food
ASASP	Association of Synthetic Amorphous Silica Producers
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BALT	Bronchus-Associated Lymphoid Tissue
BW	Body Weight
CAS	Chemical Abstract Service
cc WG	Cross cutting Working Group
CLS	Centrifugal Liquid Sedimentation
CLSM	Confocal scanning laser microscope
C_{max}	Maximum serum concentration
CPE	Cloud Point Extraction
DLS	Dynamic Light Scattering
DRC	Dynamic Reaction Cell
DRC-ICP-MS	Dynamic Reaction Cell Inductively Coupled Plasma Mass Spectrometry
EDX	Energy Dispersive X-ray Spectroscopy
EELS	Electron Energy Loss Spectroscopy
EM	Electron Microscopy
EMA	European Medicines Agency
EOGRTS	Extended One Generation Reproductive Toxicity Study
FAE	Follicle-Associated Epithelium
FAF Panel	EFSA Panel on Food Additives and Flavourings
FC	Food Categories
FEMA	Flavor and Extract Manufacturers Association
FFF	Field-Flow Fractionation
FGE	Flavouring Group Evaluation
FLAVIS	Flavour Information System database
FSMP	Food for Special Medical Purpose
GALT	Gut-Associated Lymphoid Tissue
GD	Gestation day
GFR	Glomerular Filtration Rate
GIT	Gastrointestinal Tract
GLP	Good Laboratory Practice
GNPD	Global New Products Database
HBGV	Health-Based Guidance Value
HCD	Historical Control Data
HDC-ICP-MS	Hydrodynamic Chromatography coupled to Inductively Coupled Plasma Mass Spectrometry
HDL	High Density Lipoprotein
IBO	Interested Business Operator
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
ICP-QQQ-MS	Inductively coupled plasma triple quadrupole mass spectrometry
IOFI	The International Organization of the Flavour Industry
IPCS	International Programme on Chemical Safety
IR	Infra-Red
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
JRC	European Commission's Joint Research Centre
LD	Laser diffraction
LDL	Low Density Lipoprotein
LOD	Limit of Detection
LOQ	Limit of Quantification
MALS	Multiangle Light Scattering
MCE	Mixed Cellulose Ester
MoA(s)	Mode(s) of Action
MOE	Margin of Exposure
MOP	Myeloperoxidase
MRT	Mean Residence Time
MSDI	Maximised Survey-derived Daily Intake
No	Number
NOAEL	No Observed Adverse Effect Level
NSC	Nanoscale considerations

OECD	Organisation for Economic Co-operation and Development
OHAT	Office of Health Assessment and Translation
OVA	Ovalbumin
PIL	Patient Information Leaflet
PSD	Particle Size Distribution
PTA	Particle Tracking Analysis
RoB	Risk of Bias
RP	Reference Point
SAS	Synthetic Amorphous Silica
SCF	Scientific Committee on Food
SD	Sprague-Dawley
SEM	Scanning Electron Microscopy
SOP	Standard Operating Protocol
SPC	Summary of Product Characteristics
spICP-MS	single particle Inductively Coupled Plasma Mass Spectrometry
SSA	Specific Surface Area
STEM	Scanning Transmission Electron Microscopy
TD	Toxicodynamic
TEM	Transmission Electron Microscopy
TiAb	Title and Abstract
TK	Toxicokinetic
T_{max}	Time of peak plasma concentration
WHO	World Health Organization
WoE	Weight of Evidence

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CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Commission

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NOTE

The full opinion will be published in accordance with Article 12(3) of Regulation (EC) No 1331/2008 once the decision on confidentiality will be received from the European Commission.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

Criteria for inclusion/exclusion applied to literature publications

The screening of the papers retrieved by the literature search (see Annex A) was performed using an online collaborative tool (DistillerSR[1]). This tool is designed to support the systematic reviews and allows the traceability of all the screened papers, offering also the possibility to re-iterate the process at any step.

The screening comprised two steps (i) title and abstract (TiAb) screening and (ii) full-text screening.

At the TiAb screening step, the papers retrieved by the literature search were screened for inclusion or exclusion at the level of title and abstract by two contractor's reviewers using predefined criteria. In case of doubt or insufficient information in the abstract to draw a conclusion on possible inclusion/exclusion, the papers were brought to the full-text screening for further consideration. In case of conflicting replies between the two reviewers on the possible inclusion or exclusion of papers, a conflict resolution discussion was planned.

The full text of the papers included in the first step were further screened and classified by two contractor's reviewers based on predefined criteria on the test material and type of toxicological study (see Appendix B) for further details on the classifications of studies). As for the previous step, in case of conflicting replies between the two reviewers, a conflict resolution discussion was planned.

The exclusion and inclusion criteria considered are reported in Table below. These criteria have been elaborated starting from the general question '*Does this publication provide relevant scientific information on the safety assessment of SiO_2 as a food additive?* '.

For all excluded papers, a justification was provided considering the following options:

- Not a biological/toxicological study;
- Biological/toxicological study but not relevant;
- Review/editorials/other publications;
- Test material not relevant

Table A1: Criteria for including or excluding papers retrieved by the literature search.

Criteria	Include	Exclude	In vitro
Language	Papers in English	Papers not in English	
Test material	• E 551 • Synthetic amorphous silica • SiO_2 • NM 200, 201, 202, 203, 204	• Colloidal near-spherical silica (or SiO_2) particles • Crystalline silica • Kieselguhr • Coated silica • Surface modified silica • Functionalised silica • Quartz • Silica nanocomposite • Complex of silica/silicon dioxide (with metals, other oxides, etc.) • Mesoporous silica • Fibrous silica • Engineering silica-polymer • Core-shell silica • Stober	

(Table A1 (Continued))

Criteria	Include	Exclude	In vitro
Type of study	• Primary research studies i.e. studies generating new data: ◦ In vitro ADME i.e. studies mirroring the uptake in the GIT ◦ Acellular in vitro degradation tests* ◦ In vitro other (non ADME non genotox non acellular) ◦ Ex vivo *(exception:* studies on respiratory system or skin should be excluded) ◦ In vivo toxicity and ADME studies on mammalian species ◦ In vitro/in vivo genotoxicity studies: ▪ In vitro studies in bacteria and mammalian cells ▪ In vivo studies in rodents and studies in humans ◦ Human data including studies addressing effects on microbiota in humans ◦ Occupational exposure studies focusing on oral exposure • Corrigenda should be included and labelled as 'corrigendum' and linked with the relevant reference	• Reviews (meta-analysis should be excluded but labelled with 'review or meta-analysis'), expert opinions, books and books chapter, editorials, letters to the editors, PhD thesis, extended abstracts, conference proceedings and posters • Non-biological and toxicological studies (e.g. synthesis, photocatalytic performance, soil analysis, studies on dental and bone implants) • Studies on non-mammalian species (e.g. *Drosophila*, plants, fungi, insects) • QSAR and in silico • Experimental animal studies addressing exclusively changes in microbiota (studies on animals addressing effect on microbiota and other toxicological endpoints should be included). Animal microbiota endpoints will not be considered in the assessment. • In vitro studies on respiratory system, skin or eye cells (exception: ADME, genotoxicity studies, acellular in vitro degradation text which have to be included). • Ex vivo studies on respiratory system and skin • In vivo studies that have used a non-relevant route of administration • In vivo studies performed only with not relevant test materials • Biological studies focusing on investigating on beneficial effects • Occupational exposure studies focused on inhalation or dermal exposure • Epigenetic studies	All in vitro toxicity studies except for acellular in vitro degradation studies, ADME and genotoxicity studies Studies on respiratory system, skin or eye cells should be excluded.
Exposure route	• Oral route • All routes (e.g. intravenous, intraperitoneal) for genotoxicity studies • All routes (e.g. intravenous, intraperitoneal) for ADME studies with the exception of the inhalation and dermal route	• Studies other than genotoxicity studies with exposure routes other than oral e.g. intratracheal; inhalation • Studies on ADME with inhalation and dermal exposure	
Duration	• In vivo toxicological studies with exposure equal or longer than 28 days • In vivo developmental toxicity (all types including e.g. neuro, immuno), studies on neonatal animals, genotoxicity, ADME/toxicokinetics studies independently from the exposure duration	• In vivo toxicological studies with exposure less than 28 days except for developmental toxicity, in vivo neonatal studies, genotoxicity, ADME/toxicokinetics studies that should be included	

APPENDIX B

Criteria for the classification of the toxicity studies applied to the literature search

The screening process of the papers retrieved by the literature search (see Annex A) was performed using an online collaborative tool (DistillerSR). This tool is designed to support the systematic reviews and allows the traceability of all the screened references, offering also the possibility of re-iterate the process at any step.

As first step, the papers retrieved by the literature search have been screened at level of title and abstract (TiAb) by the contractor's reviewers using pre-defined criteria (Appendix A).

The full-text papers were further screened and classified by the contractor's reviewers based on pre-defined criteria on the test material and type of toxicological study.

As a general principle, in case of doubt or insufficient information in the paper to draw a conclusion on possible exclusion, the approach taken has been to bring the publication to the following step, i.e. appraisal of relevance and reliability. In case of conflicting replies between the two reviewers on the possible inclusion or exclusion of papers, a conflict resolution discussion was planned.

To add that the exclusion criteria of TiAb are applicable also at full-text screening.

Criteria for classification test material

The full-text papers were screened to confirm relevance of the test material(s):

- the food additive silicon dioxide (E 551)
- SAS materials from the JRC repository (NM-200, NM-202, NM-203 and NM-204)
- NM-201 or SAS

At this step, studies performed with test material(s) not relevant for the assessment of E 551 were excluded.

Criteria for classification

Full-text papers considered relevant based on the text material have been then classified according to the following.

1. In vitro
   - ADME
   - Genotoxicity
   - Acellular in vitro degradation tests: in vitro gastrointestinal digestion studies and studies on the stability in lysosomal fluid
2. Ex vivo
3. In vivo
   - ADME
   - Genotoxicity
   - General toxicity (repeated dose, long term, carcinogenicity toxicity studies, combined long term and carcinogenicity studies)
   - Reproductive/developmental toxicity*
   - Neurotoxicity/Neurodevelopmental toxicity
   - Immunotoxicity
   - Human data (e.g. epidemiological)
   - Study on microbiota
   - Clinical studies on infants and young children
   - Other

*Including also studies on neonatal animals (e.g. piglet).

APPENDIX C

Advice of the cross-cutting WG on nanotechnologies on nanoscale considerations for the assessment of the study design and study results of SAS toxicity studies

C.1 | Background

The EFSA Food Ingredients and Packaging (FIP) Unit requested assistance to the cross-cutting Working Group on Nanotechnologies (hereafter ccWG Nano) in the context of the re-evaluation of silicon dioxide (E 551) as a food additive in foods for infants below 16 weeks of age and follow-up to the 2018 ANS Panel Opinion (EFSA-Q-2018-00526[54]). For this assessment, in addition to toxicological studies submitted by interested business operators (IBOs), evidence from the open literature was considered as indicated in the mandate from the European Commission. Therefore, the ccWG Nano was requested to advise on the criteria relevant to appraise toxicological studies according to nanospecific considerations for E 551, implementing the provisions of the EFSA Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles (EFSA Scientific Committee, 2021a; hereafter Guidance on Particle – Technical Requirements) and EFSA Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: human and animal health (EFSA Scientific Committee, 2021b; hereafter EFSA Guidance on Nano – Risk Assessment). The purpose of this advice is to indicate when the design of a study is adequate for hazard characterisation of nano-sized particles/aggregates in E 551. As different types/levels of information may be available depending on each study, the ccWG Nano proposed a corresponding scoring system for consideration by the requesting FIP Unit.

C.2 | Advice

C.2.1. | Considerations on particle size, dispersion generation and evaluation

An appropriate counting rule needs to be applied for risk assessment of nanomaterials in the food and feed chain and to establish the presence of small particles, including nanoparticles, in regulated food and feed product applications (EFSA Scientific Committee, 2021a, 2021b). The counting rule to measure (nano)particles for regulatory purposes in the EU encompasses isolated constituent particles as well as constituent particles within agglomerates and aggregates, which is known as the counting rule 4 (Bresch et al., 2022).[55]

Size measurement of the constituent particles requires a method such as quantitative electron microscopy that can differentiate individual constituent particles as well as constituent particles in agglomerates or aggregates (Mast et al., 2020). Both TEM- and SEM-based imaging can be applied for this purpose, provided that the limit of detection of the equipment and the imaging conditions allow accurate measurement of the constituent particles.

Methods that cannot differentiate constituent particles and agglomerates or aggregates are not suitable for reliable measurement of the constituent particle size (and constituent particle size distribution) of synthetic amorphous silica (SAS) approved as a food additive (E 551). These include techniques such as particle tracking analysis (PTA), centrifugal liquid sedimentation (CLS), laser diffraction, dynamic light scattering (DLS), multiangle light scattering (MALS), single particle inductively coupled plasma mass spectrometry (spICP-MS), field-flow fractionation (FFF) coupled with either DLS, MALS or ICP-MS (De Temmerman et al., 2014).

The data provided by IBOs show that the constituent particles in SAS used as E 551 are in the nanosize range with the majority exhibiting median minimal external dimension values from 2 to 28 nm (see Section 3.2 of this Opinion). SAS is also insoluble/very slightly soluble in water (SCCS, 2019). Therefore, in line with the EFSA Guidance on Particle – Technical Requirements (EFSA Scientific Committee, 2021a), E 551 requires risk assessment for nanomaterials following the EFSA Guidance on Nano – Risk Assessment (EFSA Scientific Committee, 2021b), to complement the conventional risk assessment conducted according to the applicable sectoral guidance.

Sufficient level of dispersion is essentially required for a proper physicochemical characterisation as well as for toxicological/toxicokinetic studies of nanomaterials as described in the Guidance on Nano – Risk Assessment (EFSA Scientific Committee, 2021b). Practical guidance for achieving an adequate dispersion of nanomaterials (also applicable to SAS), and evaluation of the dispersion stability, has been provided in the Guidance Document released by the NANoREG project.[56] It proposes a dispersion protocol using high energy to enhance deagglomeration, and minimum requirements for characterisation of and reporting on the administration matrix used in in vivo and in vitro studies. For characterisation of the dispersions, measurement of the hydrodynamic size (distribution) using DLS (and optionally CLS) is required as an easy and low cost method for evaluating the stability of dispersions and quality of dispersion in exposure media. However, while these techniques are suited for evaluating stability of dispersions, they are not suitable for measuring the constituent particle size/size distribution. In particular, methods based on light scattering, including DLS, markedly overestimate particle

[54] https://open.efsa.europa.eu/questions/EFSA-Q-2018-00526.

[55] This is the counting rule to be applied to decide whether a material is a nanomaterial according to the European Commission's Recommendation on the definition of nanomaterial (Commission Recommendation 2022/C 229/01), although as such this definition has not been adopted for the food sector.

[56] https://www.rivm.nl/sites/default/files/2018-11/NANoREG_Guidance_Document.pdf. This guidance document also provides protocols for determining effective deagglomeration and stability of dispersion.

size of the materials with polydispersed size distribution, such as SAS (De Temmerman et al., 2012; Kestens et al., 2016).[57] The stability of dispersions can also be evaluated by more advanced techniques, such as electron microscopy.

In summary, when looking for information on size distribution of particles of the test material used in a published study, it is important to consider what type of entity was measured (constituent particle, aggregate and agglomerate) and whether the data had been obtained using electron microscopy methods (SEM/TEM). Data from other methods can only provide complementary information on the size ranges of aggregates/agglomerates and stability of the dispersions of the material used in physicochemical characterisation and toxicological testing. It is also important to consider if and to which extent the sample preparation, e.g. dilution, filtration, may have impacted the measured size distribution.

C.2.2. | Considerations related to toxicity studies and safety assessment

Toxicokinetics of a material may differ between one nanomaterial and another nanomaterial with the same chemical composition but different physicochemical properties, and between nanomaterial forms and conventional (bulk) form of the same material, if they exist. For safety assessment, information on ADME of SAS materials is therefore needed, e.g. gastrointestinal uptake, tissue distribution and potential for accumulation (EFSA Scientific Committee, 2021b).

A suitable analytical methodology should be carefully selected for ADME studies, for demonstrating internal exposure by tracking biodistribution and tissue particle deposition (in vivo studies), or cellular uptake (in vitro studies). Quantitative and sensitive methods to determine particulate SAS in biological matrices are currently not mainstream. Therefore, often measuring total silicon is used as a proxy for SAS. In such cases, selection of sensitive methods, checking of potential analytical interferences and the use of adequate controls are needed. Furthermore, background exposure to dietary Si forms (i.e. Si present in feed of laboratory animals) has to be evaluated and considered in the interpretation of the results from in vivo animal studies (Aureli et al., 2020; Brand et al., 2021). Aureli et al. (2020) highlighted that analytical techniques based on total Si determination in biological samples can confidently be used if certain aspects are appropriately dealt with: (i) contamination due to the ubiquitous presence of silicon in reagents, in labware and analytical equipment, (ii) the existence of a substantial level of biological background of silicon due to natural presence in tissues, (iii) the conversion of (chemically resistant) silica particles into soluble silicon (e.g. by using hydrofluoric acid), (iv) the severe spectral interferences affecting detection of silicon by ICP-MS. The lack of reference materials in a biological matrix for checking the accuracy of measurements may further complicate this undertaking (Aureli et al., 2020). Therefore, when ICP-MS is used as the analytical technique for determining SAS via Si detection, reliable results can be obtained through removal of spectral interferences by the use of either ion-molecule chemistry (i.e. reaction cells) (Aureli et al., 2012; Aureli et al., 2020), ICP-MS/MS (Aureli et al., 2015; Boudard et al., 2019; Gonzalez et al., 2015; Tassinari et al., 2020) or high-resolution ICP-MS (Peters et al., 2020).

EM-based methods, although not providing quantitative data, are suitable to demonstrate internal exposure of cells and tissues to E 551, and to locate the presence of silica particles in cells and tissues, provided that they are coupled to another suitable analytical method, such as energy dispersive X-ray spectrometry (EDX) or electron energy loss spectroscopy (EELS), which allows identification of silica particles by their elemental composition. Demonstrating that cells are not exposed to E 551 particles through the absence of silica particles by EM alone is not conclusive because EM only allows for checking the presence of particles in a very small amount of tissue that may not be representative of the tissue/organ. On the other hand, positive detection of silica particles in comparison with control samples will provide a conclusive proof for demonstrating intracellular exposure.

Ensuring that SAS nano-sized particles/aggregates are properly dispersed, and their extensive agglomeration (and possible associated decreased bioavailability) has been prevented, is important when in vitro and in vivo studies are undertaken (see Section 2.1). Gastrointestinal absorption is expected to be affected by the degree of agglomeration of nanomaterials. The degree of agglomeration often increases with concentration/dose. In the case of E 551, increased concentration also leads to increased viscosity due to gel formation. The formation of larger sized agglomerates may thus lead to a relationship between dose and internal exposure that is not linear. For instance, van der Zande et al. (2014) reported a non-linear oral absorption rate of SAS in an oral in vivo rat study where the test material was incorporated in the diet. A higher oral absorption of SAS was found at the lowest dose (100 mg/kg bw per day), regarded as more relevant to human dietary exposure, compared to higher doses tested (500–2500 mg/kg bw per day) (van der Zande et al., 2014). These issues have been discussed in detail in Brand et al. (2021). Therefore, although E 551 is not a highly agglomerative material in a water-based medium as compared to, e.g. titanium dioxide, considerations relating to a high degree of agglomeration are still relevant when high doses/concentrations are tested without proper dispersion. Hence, in both ADME and toxicity studies, to the extent possible, the relationship between dose and tissue concentrations should be assessed.

In line with the above, specific considerations on the degree of agglomeration of the tested material is essential for assessing the results of toxicokinetic and toxicity studies. If the test material was not properly dispersed before testing, and it can be expected to be mainly comprised of large agglomerates, the results may only be relevant for assessing local effects of large agglomerates. However, the results from such a test will not cover the full extent of potential uptake and barrier crossing of nano-sized particles/aggregates in human gastrointestinal tract, and of translocation of nanoparticles

[57] The inherent bias of DLS (and MALS) towards the larger sizes when materials with a high size polydispersity are analysed also implies that these methods are not suitable to representatively measure and demonstrate the absence of relatively small particles, including nanoparticles. Further clarification on the 'the use of Dynamic Light Scattering (DLS) and other light scattering methods for characterisation of particle size distribution' is provided in the EFSA Webpage on Nanotechnology https://www.efsa.europa.eu/en/topics/topic/nanotechnology.

to other organs with consequent systemic effects. It should also be noted that most organs and organ systems are not structurally and functionally fully mature at birth but neonates have a mature-type intestinal epithelium. The absorption of small or nanoparticles may be different in neonates compared to adults and once absorbed, the distribution and health effects may be different due to e.g. changes in body composition, organ function, enzyme development (EFSA Scientific Committee, 2017). Furthermore, nanoparticles may have adverse effects on the intestinal epithelium layer, including impaired barrier function and immune damage (Issa et al., 2022).

In summary, when looking for information on the toxicity of SAS in a study, it is important to assess: (i) whether the test material was properly dispersed before administration/testing and characterisation thereof was performed on the matrix of administration, (ii) the dose-dependency of any toxicity and toxicokinetic information, considering if suitable analytical method(s) were used to characterise (qualitatively and quantitatively) the chemical composition and particulate nature of the test material, (iii) that the doses used were not too high to lead to agglomeration and gel formation.

C.2.3. | Scoring for nanoscale considerations

Materials in particulate form are known to have a certain degree of surface free energy because of the inward pull of the atoms/molecules at the particle surface. Because of this extra potential energy, particles tend to stick to surfaces and to other particles. This tendency becomes more pronounced when the particle size is decreased and the surface area is increased. At the nanoscale, particles have relatively larger surface areas and higher surface free energies compared to larger sized particles, and hence have a greater tendency to adhere to other particles to form agglomerates (Balakrishnan et al., 2010; Simon & Joner, 2008).

In this regard, it is important that particle agglomeration is not confused with particle aggregation.[58] Whilst small/nano particles tend to readily agglomerate in a dispersion due to faster Brownian motion, particle aggregates are generally only formed during a manufacturing process – especially when the use of high energy (e.g. laser ablation, pyrogenesis) is involved. This leads the constituent particles to be strongly bound, e.g. through metallic or covalent bonds. Particle aggregates are thus very difficult to de-aggregate and are not expected to release constituent particles under normal physiological or biological conditions. Compared to this, particles in an agglomerate are only loosely-held together by weak van der Waals forces or electrostatic interactions, and can relatively easily de-agglomerate to release nanoparticles under certain conditions, e.g. due to a physical force or a change in pH or ionic concentration (Bruinink et al., 2015; OECD, 2012; Rauscher et al., 2023). It is, therefore, important that particle agglomerates are considered as dynamic interchangeable entities that can de-agglomerate and release constituent particles and small-sized aggregates under various physical and biological conditions (Peters et al., 2012). Therefore, for risk assessment of nanomaterials, dispersion of agglomerates is necessary to mimic consumer intake regarding the size of particles in food items containing E551. This is also important because smaller particles (constituent, aggregates and agglomerates) are more likely to cross membrane barriers than larger sized particles. This can lead to internal exposure of an in vitro test system or in vivo laboratory animal to insoluble and potentially surface-reactive particles that might interfere with cellular functions through interaction with various biological entities or through prolonged release of ions (Bruinink et al., 2015; De Matteis et al., 2015). To be able to observe any small/nano particle related effects – which in the absence of dispersion might be missed because of the inability of larger-sized particle clusters to penetrate membrane barriers – a toxicological study must be carried out on the test material that has been adequately dispersed. The absence of toxicological effects noted in a study, in which the test material was not subjected to adequate dispersion, may not be useful to conclude on the potential toxicity of nano-sized particles.

C.2.3.1. | Specific considerations for SAS

The EFSA Guidance on Nano – Risk Assessment (EFSA Scientific Committee, 2021b) recommends that toxicity testing should be conducted with a material with the lowest level of agglomeration to which consumers will be exposed following normal use conditions, and appropriate dispersion should be used to de-agglomerate any agglomerated particles to cover consumer exposure to small or nano-sized particles. The level of agglomeration of nanoparticles is also dependent on the particle concentration and can be expected to increase with the concentration/dose (Gudkov et al., 2020; van der Zande et al., 2014). As a rule of thumb, the higher the concentration, the higher the chances of agglomeration of nanoparticles. Adequate dispersion and characterisation of the SAS dispersion become even more important because of potential gelation with increasing concentrations.

The EFSA Guidance on Nano – Risk Assessment (EFSA Scientific Committee, 2021b) recommends that '*studies conducted at high doses (for in vitro tests all concentrations above 100 μg/mL; for in vivo studies, all doses above 50 mg/kg body weight (for liquid forms) or 100 mg/kg body weight (when incorporated in the food matrix)), without information on dispersion and stability or confirmation of cellular/tissue exposure are insufficient for the hazard identification and characterisation of nanoparticles, as well as for the hazard identification and characterisation of the fraction of nanoparticles of conventional materials*'.

Accordingly, this indication of concentrations/doses was also used for the appraisal of nano-specific aspects of toxicological studies on titanium dioxide (E171) (EFSA FAF Panel, 2021), and these threshold concentrations/doses were calculated

[58]For details on the difference between aggregates and agglomerates refer to the ISO definition (https://www.iso.org/obp/ui/en/#iso:std:iso:ts:23302:ed-1:v1:en) (ISO, 2015).

on the basis of the maximum concentration of TiO_2 that could be dispersed according to the protocols (e.g. EU-funded projects ENPRA, Nanogenotox, NANoREG) for preparation of suspensions for in vivo toxicological studies for the hazard assessment of small particles.[59] As noted above (see Section 2.2), with regard to SAS, evidence of a non-linear oral absorption rate was found in an oral in vivo rat study with incorporation of the test material in the animal diet (feed matrix): a higher oral absorption fraction for SAS was observed at the lowest dose (100 mg/kg bw per day) that is more relevant to human dietary exposure, compared to the higher doses tested (500–2500 mg/kg bw per day) (van der Zande et al., 2014). These issues have been discussed in detail in Brand et al. (2021) who, quoting several studies on SAS, have noted that '*it is believed the degree of agglomeration may increase at higher doses, which might explain why studies at high doses fail to find effects*'. It is also known that increasing concentrations of SAS materials in aqueous media tend to result in a gel-like matrix. The onset of gel formation depends not only on the SAS concentration but also on specific conditions, such as the presence of divalent ions in the aqueous medium. The onset of gelation corresponds to the higher dose range for toxicity studies. For example, van der Zande et al. (2014) characterised SAS and NM-202 after in vitro digestion. They observed that '*the intestinal content of the mid/high-dose groups* [corresponding to 500, 1000 and 2500 mg/kg/day] *had stronger gel-like properties than the low-dose groups* [corresponding to 100 mg/kg/day], *implying low gelation and high bioaccessibility of silica in the human intestine at realistic consumer exposure levels*' (van der Zande et al., 2014).

In view of the evidence above and considering that the impact of the degree of agglomeration on absorption is still largely unknown, the upper concentrations/doses indicated in the Guidance on Nano – Risk Assessment (i.e. for in vitro tests: all concentrations above 100 µg/mL; for in vivo studies: all doses above 50 mg/kg body weight (for liquid forms) or 100 mg/kg body weight (when incorporated in the food matrix)) are proposed as a conservative but pragmatic approach for ensuring that the toxicokinetic and toxicological studies have covered nano-sized particles/aggregates. However, in vivo studies with higher doses than those mentioned above can also be considered adequate provided that proper dispersion of the test item has been demonstrated by the authors of the studies.

Table C.1 presents the criteria proposed for scoring the capacity of the study for addressing the possible hazard of nano-sized particles/aggregates.

[59]The concentration of the stock dispersion in these internationally agreed generic dispersion protocols is 2.56 mg/mL (e.g. EU-funded Projects ENPRA, NANOGENOTOX, NANOREG). Considering the maximum volume that can be administered by gavage (20 mL/kg bw divided over twice a day i.e. OECD TG 408) or, for administration via the animal diet, the incorporation of 1 mL of dispersion in 2 g of food and the factors for converting concentrations in feed into daily doses in sub-chronic studies of the EFSA Guidance on default values (EFSA Scientific Committee, 2012), the upper concentrations/doses are estimated.

TABLE C.1 Criteria for the appraisal of the nanoscale considerations for synthetic amorphous silica (E 551).

	Criteria for scoring of in vitro/in vivo studies for adequacy and relevance to risk assessment of E 551	Considerations for interpreting the results for the hazard characterisation of nano-sized particles/aggregates
Score 1	At least one of the following criteria is met: • Verified SOP for dispersion of the test item (e.g. ISO, OECD and EU-funded Projects ENPRA, Nanogenotox and NANoREG)[60] • Sonication energy applied 600–2500 J/mL plus confirmation of stability for at least 30 min (or until last administration) using one of the methods described in Section 3.2 of the EFSA Guidance on Particle – Technical Requirements • Confirmation of adequate level of dispersion and dispersion stability (e.g. by DLS, CLS, zeta potential measurement)[61,62,63] of the matrix that is administered/used in in vivo/in vitro studies. • Use of demonstrably effective dispersing agents or surfactants with a proper justification (and inclusion of solvent control) • In case of administration via feed diet: the level of agglomeration in feed should be comparable to the level of agglomeration in commercial food products. How the food additive is used/incorporated in the final food (e.g. to modulate viscosity, anticaking, spray-dry) should be also considered to address whether the level of dispersion in the test system (in vitro *or* in vivo) is higher than/or mimics that can be expected in the food • Confirmation of cell/tissue exposure during execution of the test (e.g. ICP-MS or ultrastructural localisation by EM, enhanced darkfield microscopy); including evidence that the particles correspond to the test material	The evaluator can consider both positive and negative results as relevant and reliable from the perspective of assessing nano-sized particles/aggregates Note: If the score is based on confirmation of internal exposure, when assessing the results, the evaluator should consider in the WoE and uncertainty analysis whether the confirmation is only qualitative (e.g. EM supported by verification of Si in the particles) or if, as preferable, there is a quantitative assessment (e.g. Si measurement) of the level of internal exposure at each dose/concentration
Score 2	None of the criteria above fulfilled, but there are some indications in the study design and, although incomplete, some information on dispersion as used in the in vivo/in vitro studies, is presented	The relevance of the results for assessing nano-sized particles/aggregates may be affected by the degree of agglomeration because larger size of agglomerates may be present. Also, since increasing concentrations lead to more particle agglomeration, the assessor should check the reliability of the dose–response relationship case by case, and whether the study provides a 'a conclusive observation'

[60]The NANOGENOTOX and ENPRA protocols, subsequently adopted also in NANoREG, prescribe a stock concentration of 2.56 g/L. Therefore, the use of an upper concentration greater than this value is to be considered not compliant with these dispersion protocols. From the above it follows that these protocols can only be applied to administer in vivo doses up to 50 mg/kg body weight (for liquid forms) or 100 mg/kg body weight (when SAS is incorporated in the food matrix).w

[61]Degree of agglomeration assessed for each dose/concentration using methods described in Sections 3.2 and 3.3 of the EFSA Guidance on Particle – Technical Requirements.

[62]DLS can only be used to get information on the agglomeration state if the hydrodynamic diameter is small (e.g. smaller than 250 nm). In general, DLS can only be used to assess the stability of a dispersion, i.e. a dispersion is stable when no or limited changes in time in the hydrodynamic diameters are observed.

[63]Information about the particle size in intestinal fluid conditions at the relevant administered dose can be used to conclude that a material is well dispersed, provided that the analytical measurement does not introduce bias (e.g. dilution before analytical measurements may lead to deagglomeration).

Table C.1 (Continued)

	Criteria for scoring of in vitro/in vivo studies for adequacy and relevance to risk assessment of E 551	Considerations for interpreting the results for the hazard characterisation of nano-sized particles/aggregates
Score 3	When criteria 1 and 2 are not met and no clear evidence of adequate dispersion is available (e.g. magnetic stirring, vortexing, hand-shaking and ultrasound bath used), but concentrations are in vitro below or at 100 μg/mL, in vivo below or at 50 (liquid) or 100 (food matrix) mg/kg bw (section 7.3 of the EFSA Guidance on Nano – Risk Assessment)	The relevance of the results for assessing nano-sized particles/aggregates cannot be verified (i.e. cannot be confirmed but cannot be excluded). This situation creates uncertainty regarding the capacity of the study results to cover the nano-sized particles/aggregates. Specifically, the evaluator should consider that negative results may be due to the lack of exposure to nano-sized particles/aggregates In consideration of E 551 physicochemical properties, score 3 can also be applied to the following situations: The dose levels mentioned are achieved with a suspension of up to 2.6 mg/mL (as recommended in the Nanogenotox/NANoREG protocols) but the recommended procedure for sonication has not been used for particle dispersion. Instead, other methods have been used, such as magnetic stirring, vortexing, hand-shaking and ultrasound bath, without clear evidence of adequate dispersion. It needs to be considered that the level of agglomeration of SAS at such a concentration can be higher compared to when probe sonication was used. It is therefore expected that some 'absorbable' particles (< 250 nm) are present in the absence of the dispersion obtained by sonication. If the information on aggregation/agglomeration status has not been provided, the risk assessor should be aware that dose–response relationships at these doses/concentrations (i.e. in vitro above or at 100 μg/mL, in vivo above or at 50 (liquid) or 100 (food matrix) mg/kg bw) will probably not be linear. Higher doses will likely show a higher degree of agglomeration compared to lower administered doses
Score 4	When criteria 1, 2 and 3 are not met and only high concentrations/doses are used (i.e. in vitro above or at 100 μg/mL, in vivo above or at 50 (liquid) or 100 (food matrix) mg/kg bw) without further information on dispersion and stability or verifiable information that the study design simulates actual consumer exposure to E 551 The score also applies when there are observations confirming substantial precipitation, gel formation or increase in viscosity	When a study is designed for testing only high concentrations/doses, and lacks specific considerations on dispersion, presence of large agglomerates of particles must be considered. The results may still be informative for larger particles and large agglomerates, but not for the nano-sized particles/aggregates. In the case of in vivo studies, such a study design should trigger a high likelihood for reduced bioavailability of nanoparticles due to extensive agglomeration. Negative results are therefore not informative for assessing consumers' exposure to the nano-sized particles/aggregates. In case of positive results, the evaluators should consider that the dose may not represent the actual fraction of nanoparticles available for absorption. For in vitro studies, a similar concern should be considered for negative results. For positive results, artefacts due to local effects of precipitation should be considered

APPENDIX D

Approach for the appraisal of the toxicity studies

Background

The validity of a study with respect to risk assessment has two dimensions. The first dimension is relevance of the endpoint(s) used for the risk assessment. This is often described as 'external validity'.

The second dimension is reliability, which considers systematic error (accuracy, risk of bias) and random error (imprecision). Risk of bias (or internal validity) is defined as 'the extent to which the design and conduct of a study are likely to have prevented bias'. Risk of bias relates to the propensity of a study to be affected by systematic error. Biases can operate in either direction and can lead to underestimation or overestimation of the true intervention effect.

The Panel considered and agreed on a set of criteria to be followed for the evaluation of the evidence that will be included in the scientific opinion, compatible with the timeline and the scope of the mandate. The criteria cover the two dimensions of:

- Relevance
- Internal validity

In the current assessment for E 551, two main research questions were considered:
1Q. Does the food additive E 551 raise a concern for genotoxicity?
2Q. Does exposure to the food additive E 551 **through the diet** raise a health safety concern?
The latter question defines the overall boundaries of this risk assessment.

Appraising relevance (external validity) of the studies

As genotoxicity is considered a standalone endpoint, the EFSA cross cutting (cc)WG genotoxicity developed a procedure on the approach for assessing reliability and relevance of genotoxicity studies, including nanospecific considerations, according to the harmonised approach for reporting reliability and relevance of genotoxicity studies (EFSA, 2023).[64]

To facilitate an efficient assessment of the literature database, the first step was to include only literature if it was considered relevant to the following key subquestions and exclude literature if not relevant:

- Does the literature contribute to the knowledge on ADME of E 551 (absorption, distribution, metabolism (if any), route and rate of excretion, half-life, accumulation)?
- Does E 551 cause any adverse effect on general toxicity endpoints (e.g. body weight changes, organ weight changes, haematology, clinical chemistry)?
- Does E 551 cause any adverse effect on the GIT?
- Does E 551 cause any adverse effect on the liver?
- Does E 551 cause any adverse effect on the spleen and (developing) immune system?
- Does E 551 cause any adverse effect on the reproductive system, or does it affect development?
- Does E 551 cause any adverse effect on the (developing) nervous system?
- Does E 551 cause any adverse effect on other organs/systems?
- Does E 551 promote or cause tumours (carcinogenicity) by the oral route?
- Are there changes of intestinal microbiota after intake of E 551 related to adverse health effects?

Screening for relevance (external validity)

An initial exercise of screening for relevance was conducted by a contractor on the publications retrieved from the literature, in consultation with the EFSA ccWG nanotechnology (Appendix C). The aim of this initial step was to exclude from further screening those studies conducted with test material not relevant for E 551 and with routes of exposure or test systems deemed as clearly non-relevant.

Taking into account the advice from the EFSA ccWG Nanotechnology, the criteria for inclusion and the exclusion were defined (Appendix A. Toxicological studies performed with the food additive silicon dioxide (E 551), SAS silicon dioxide (SiO_2), NM 200–204 were included.

[64]EFSA (European Food Safety Authority), Andreoli, C., Aquilina, G., Bignami, M., Bolognesi, C., Crebelli, R., Dusinska, M., Gürtler, R., Louro, H., Marcon, F., Nielsen, E., Schlatter, J., Vleminckx, C., Astuto, M. C., Nathanail, A. V., & Benford, D. (2023). Harmonised approach for reporting reliability and relevance of genotoxicity studies. EFSA Supporting Publication, 2023, EN-8270. 10.2903/sp.efsa.2023.EN-8270.

The WG agreed to focus on the in vivo toxicity studies to identify potential adverse effects. In vitro studies are parked awaiting the outcome of the in vivo studies assessment. In case a potential adverse effect in vivo has been identified the in vitro studies are screened to identify studies which could support MOA considerations.

Appraisal for confirmation of relevance of toxicity endpoints

According to the agreement to focus the assessment first on the in vivo toxicity studies, an appraisal for relevance was carried out by Expert members of the FAF WG Follow up Tox.

A decision was taken to classify relevant papers in the following categories, according to a limited set of criteria:

- **Relevant:** Studies designed in such a way that would allow their use for identifying a reference point (RP) that could be used for deriving a health-based guidance value (HBGV) and studies that could provide supporting information to the risk assessment.
- **Not relevant:** Route of administration different from oral (except for toxicokinetic studies where intravenous studies can provide relevant information);

The appraisal for relevance was conducted in parallel independently by two experts and when a conflict in classification of the study was identified, ad hoc discussion between the reviewers took place prior to a final agreed classification.

In a second step, if a potential adverse effect was identified in the in vivo studies, results from the in vitro studies have been considered in the assessment if the study investigates relevant endpoints related to effects observed in the in vivo studies:

- using relevant experimental model systems.
- providing information on mode of action (e.g. cellular, or molecular mechanism)
- providing quantitative information on concentration/dose response effects and the concentrations used are relevant (e.g. less or equal than 100 μg/mL (see the Advice by the ccWG Nanotechnologies, Appendix C)

Appraising the internal validity of evidence

In vivo studies that were confirmed as relevant were individually appraised for their internal validity.

For the reproductive and developmental toxicity studies, the appraisal of the internal validity and reliability have been conducted at the same time, noting that most of the studies will be considered relevant hence it was considered more efficient to conduct the appraisal in one go.

The original Office of Health Assessment and Translation (OHAT) tool is based on sets of questions grouped under the following bias domains:

- Selection
- Performance
- Attrition/exclusion
- Detection
- Selective reporting
- Other threats to internal validity

In the present assessment, the OHAT Risk of Bias Rating Tool for Human and Animal Studies[65] has been modified and used for the appraisal of both animal and human studies.

Reliability regarding the specific 'toxicity' endpoint

Publications were allocated to two experts, members of the FAF WG Follow-up TOX with expertise relevant to the endpoint reported. The screening step for internal validity was also conducted in parallel independently by two experts and when a conflict in classification of the study was identified, ad hoc discussion between the reviewers took place prior to a final agreed classification and a final rating was assigned to the endpoint(s) investigated through consensus.

The following rating was used to indicate whether the design and conduct of the study supported the association (or the lack of it) between the exposure to the test material and the reported outcome:

1. Yes, only minor or no limitations (Risk of bias, RoB 1)
2. Overall yes, with some limitations (Risk of bias, RoB 2)
3. Ambiguous, there are flaws that may hamper evaluation of the association (Risk of bias, RoB 3)
4. No, there are major flaws (Risk of bias, RoB 4)

[65] https://ntp.niehs.nih.gov/ntp/ohat/pubs/riskofbiastool_508.pdf.

The rating assigned by the Experts took into account the following aspects of the design and performance of the studies, borrowed from the OHAT risk of bias tool.

Table D1: Description of the elements considered for the rating of the reliability for each bias domain.

Bias domain	Questions	Elements considered for the rating of reliability	Notes on the questions/decisions taken
1. Selection	Did each animal have an equal chance of being assigned to study groups (randomisation of exposure)?	Randomisation should be reported but detailed methods are not critical	**Note 1** Randomisation when data are available for more generations (studies on animals): – In case of a developmental study where the pups are assigned to different level of exposure or different testing, the whole randomisation process, from mothers to pups, including litter effects, needs to be considered. – In case of carcinogenicity studies with pre-natal exposure: normally randomisation is reported only for the parental generation and not for the pups. This was agreed to be considered acceptable only for the carcinogenicity studies. **Note 2** This question refers to the dosing and treatment part of the experiment (i.e. that the people responsible for the maintenance of the animals may know or not which ones are the controls and which ones are the dosed) and **not to the outcome measurement** (the question that refers specifically to the blinding of the outcome assessor is reported below in this form under question of #4) Acceptable methods, used to ensure allocation concealment, include sequentially numbered treatment containers of identical appearance or equivalent methods
2. Performance	Were there systematic differences in the environmental conditions and handling of animals among the study groups?	It is assumed that, unless stated otherwise, experimental conditions and handling of animals were identical across groups	
3. Attrition/Exclusion	Is the number of animals reported in the results smaller than the number of animals given in the method section? Were the missing outcomes explained? Could the missing outcomes impact the effect estimates? How were they accounted for in the results?	Exclusion of individuals or animals from analyses should be clearly reported and outliers identified with appropriate statistical procedures	**Note 3** If the loss of the animals is not acceptable, this should be considered as an important flaw. If the loss is different for different endpoints, this raises concern and then the experts should report this in their appraisal. Experts should evaluate, depending on the study (group size/number of animals, endpoint), whether missing outcomes, even if explained, may impact the effect estimates.

Table D1 (Continued)

Bias domain	Questions	Elements considered for the rating of reliability	Notes on the questions/decisions taken
4. Detection	Were there systematic differences between experimental and control groups with regard to how outcomes are assessed? Were the methods used to assess outcomes reliable? Can we be confident in the outcome characterisation?	The experts will look/looked at the acceptability of the method used (e.g. gold standard, valid and reliable, insensitive), at the length of time at which the outcome was assessed (same across study groups). Allocation concealment during outcome assessment should be reported but it is critical only for behavioural testing and quantitative histopathology. With respect to the blinding, it is noted that lack of adequate blinding of the assessors may cause bias of the results, which is more likely to apply to subjective outcome measures (e.g. a behavioural outcome rated by a researcher). For some outcomes, particularly histopathology assessment, outcome assessors are not blind to study group as they require comparison to the control to appropriately judge the outcome, but additional measures such as multiple levels of independent review by trained pathologists should have been applied minimise this potential bias.	**Note 4** For manually scored endpoints, blinding is important, whereas for non-manually scored endpoints is less important In case that the experts believe that the adequate blinding procedures were not followed this would have an impact on the study validity. More in details, WG agreements: – Behavioural testing: blinding is needed (even if endpoints are recorded automatically; due to possible handler effects). If blinding is not adequate, it is considered a major flaw. – Glucose tolerance test (GTT): blinding is needed. – Body weight: blinding is not needed. – Blinding for organ extraction may be needed on case by case, in particular from small organs, difficult for preparation. – If blinding is not adequate, it is considered a major flaw for the relevant organ. – Histopathology: quantitative morphometry requires blinding. Confirmation of histopathological findings should be blinded. – Manual sperm analysis: blinding is needed. **Note 5** Number of animals, the WG agreed: – to downgrade the scoring when number of animals used in the study are lower than recommended in OECD Test Guidelines. – The minimum number of animals should normally be 5 per sex. Justification should be provided for testing only one sex. If not present, this will represent a limitation.
Selective reporting	Were all measured outcomes reported?	It is acknowledged that this element is difficult to assess with confidence for most studies unless the study protocol is available. In the majority of the cases, this element can only be assessed by comparing the 'Abstract', the 'Materials and methods' and the 'Results' sections of the publications.	**Note 6** In case not all measured outcomes are reported, it can be considered a limitation, depending on the endpoint it could be a flaw.
Other threats to internal validity?	Were statistical methods appropriate? Was the research question clear?	One of the common statistical issues identified has been reporting of statistical tests that require normally distributed data (e.g. *t*-test or ANOVA) without reporting that the homogeneity of variance was tested or confirmed.	**Note 7** The choice of statistical analyses will depend on the type of study and the nature of the endpoints measured The WG agreed also that: – Statistical testing comparing several dose levels/arms compared to control without preceding positive ANOVA is not appropriate. The WG agreed to downgrade the score in these cases. – For reproduction studies the unit of statistical analysis is the litter: if this is not considered, this is a major flaw. – Exclusion of outliers (e.g. organ weights processed as a covariate of body weight): statistical analysis to identify outliers should be reported.

The rating was based on experts' judgement, with a narrative description of the elements considered for the overall rating. This information was managed and recorded using an online tool for all type of studies (DistillerSR software) with the exception of ADME studies and acellular degradation studies, which have been evaluated by the experts narratively since the scoring elements related to the aspects of the design and performance of the studies were not applicable for such studies.

Only studies, considered sufficiently reliable (RoB 1 and 2), were included in the assessment. Studies of RoB 3 could be used as supporting evidence in selected cases with a justification. Studies of RoB 4 were not considered in the assessment.

APPENDIX E

Exposure assessment to toxic elements from the use of silicon dioxide (E 551) as a food additive

Analytical data on the presence of toxic elements in different samples of fumed, precipitated SAS and SAS gel (E 551) using different methodologies (i.e. partial digestion, full digestion and HCl extraction) were submitted (Documentation provided to EFSA No. 2). Some variation in the measured levels of the toxic elements is noted depending on the sample preparation method. Higher contents of toxic elements are reported for some samples analysed after full digestion. No major difference is observed between the result of the analysis of lead, mercury and arsenic between the different types of SAS. A prominent difference is observed in the case of aluminium between fumed and SAS produced by the wet process.

The FAF Panel considered that the analysis of impurities after HCl extraction better reflects the bioavailable content of the impurities and considered this method sufficiently conservative to assess the exposure to those impurities from the use of E 551 as a food additive. Full digestion of silicon dioxide requires harsh methods with hazardous hydrofluoric acid in specialised analytical laboratories. Full digestion makes the whole toxic element content measurable, while the HCl extraction covers the soluble content of the toxic element obtained under the specific extraction conditions. Consequently, the full digestion method reveals the total content of toxic elements in the product and represents the upper limit that could be obtained by extraction. Therefore, if the results obtained for toxic elements using the full digestion method are below a defined limit, they will also be below the defined limit established using the HCl extraction method.

The potential exposure to impurities from the use of silicon dioxide (E 551) can be calculated by assuming that the impurity is present in the food additive up to a limit value, and then by calculation pro-rata to the estimates of exposure to the food additive itself.

With regard to the dietary exposure to the food additive for the general population, the Panel considered the exposure calculations performed at the time of the re-evaluation (EFSA ANS Panel, 2018). The ANS 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. For the current assessment, the rounded highest exposure levels for the mean and 95th percentile among the different population groups were considered, i.e. 18 and 50 mg/kg bw per day, for children (Table E.1).

TABLE E.1 Summary of dietary exposure to silicon dioxide (E 551) from their use as a food additive in the refined non-brand-loyal scenario exposure scenario, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day) (EFSA ANS Panel, 2018).

	Infants (12 weeks to 11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Refined non-brand-loyal scenario estimated exposure assessment 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

Regarding the estimated exposure to silicon dioxide (E 551) from its use as a food additive in FC 13.1.1 and FC 13.1.5.1 for infants below 16 weeks of age, a scenario has been calculated using maximum level reported by IBO (Table 6, Section 3.3.2). The mean and high level consumption were 2 and 3 mg/kg bw per day, respectively. While considering the mean of the levels reported by IBO, the highest mean and high level consumption were 0.8 and 1.1 mg/kg bw per day, respectively.

The level of toxic elements in E 551 combined with the estimated intakes of E 551, presented in Tables E.1 and 6, could result in an exposure which can be compared with the following reference point for the toxic elements (Table E.2).

TABLE E.2 Reference points for toxic elements potentially present in silicon dioxide (E 551).

Impurity HBGV/RP	Basis/reference
Lead (Pb)/0.5 µg/kg bw per day ($BMDL_{01}$)	The reference point is based on a study demonstrating perturbation of intellectual development in children with the critical response size of 1 point reduction in IQ. The EFSA CONTAM Panel mentioned that a 1 point reduction in IQ is related to a 4.5% increase in the risk of failure to graduate from high school and that a 1 point reduction in IQ in children can be associated with a decrease of later productivity of about 2%. A risk cannot be excluded if the exposure exceeds the $BMDL_{01}$ (MOE lower than 1) EFSA CONTAM Panel (2010)
Mercury (Hg)/4 µg/kg bw per week (TWI)	The HBGV was set using kidney weight changes in male rats as the pivotal effect. Based on the $BMDL_{10}$ of 0.06 mg/kg bw per day, expressed as mercury, and an uncertainty factor of 100 to account for inter and intra species differences, with conversion to a weekly basis and rounding to one significant figure, a TWI for inorganic mercury of 4 µg/kg bw per week, expressed as mercury was established EFSA CONTAM Panel (2012)

(Continued)

Impurity HBGV/RP	Basis/reference
Inorganic arsenic (iAs)/0.06 μg/kg bw ($BMDL_{05}$)	The reference point is based on a benchmark dose lower confidence limit (BMDL05) of 0.06 μg/kg bw per day identified for skin cancer. The reference point is considered to cover lung cancer, bladder cancer, skin lesions, ischemic heart disease, chronic kidney disease, respiratory disease, spontaneous abortion, stillbirth, infant mortality and neurodevelopmental effects. An MOE of 1 would correspond to the exposure level that is associated with a 5% increase relative to the background incidence for skin cancer, based on the available data'. An MOE of 1 raises a health concern Because there are no precedents in EFSA for identification of an MOE of low concern, when using a BMDL derived from human cancer data the CONTAM Panel decided not to determine a value for an MOE of low concern EFSA CONTAM Panel (2024)
Aluminium (Al)/1000 μg/kg bw per week (TWI)	The HBGV is based on the combined evidence from several studies in mice, rats and dogs that used dietary administration of aluminium compounds. In these studies the lowest no-observed-adverse-effect levels (NOAELs) for effects on neurotoxicity, testes and embryotoxicity, were reported at 30, 27 and 100, and for effects on the developing nervous system, between 10 and 42 mg aluminium/kg bw per day, respectively. Based on the combined evidence from the above-mentioned studies, the EFSA AFC Panel established a TWI of 1000 μg Al/kg bw per week EFSA (2008)

Abbreviations: $BMDL_{05}$, benchmark dose (lower confidence limit); bw, body weight; HBGV, Health-based guidance value; MOE, margin of exposure; RP, Reference point; TWI, tolerable weekly intake.

The risk assessment of toxic elements helps inform whether there could be a possible health concern if these impurities would be present at a specific limit value in the food additive. The assessment is performed by calculating the MOE (margin of exposure) by dividing the reference point (e.g. BMDL Table E.2) by the exposure estimate (Tables E.1 and 6, Section 3.3.2), or by estimating the contribution of the use of E 551 to the HBGV (expressed as percentage of the HBGV). It is considered that any mercury or arsenic in the samples correspond to the elements in the inorganic form rather than organic forms. Consequently, for the comparison, the HBGV for inorganic mercury and the RP for inorganic arsenic were used for the comparison (Table E.2).

Considering the results of the analysis by HCl extraction (Table 2, Section 3.2.2), concentration data were reported for lead in 11 out of the 17 analysed samples, up to 2.1 mg/kg (LOQ 0.1 mg/kg). Mercury and arsenic were reported below the LOQ, i.e. 0.05 and 0.1 mg/kg, respectively (Documentation provided to EFSA No. 7).

Considering the results of the analysis of aluminium by HCl extraction method (Table 2, Section 3.2.2), the content of aluminium in different E 551 range from below 1 to 420 mg/kg (Documentation provided to EFSA No 22).

The Panel performed the risk assessment that would result if the toxic elements were present in E 551 at: (i) the existing maximum limit in EU specifications; (ii) from the data reported with the HCl extraction method, the rounded up highest measured value of the level of the toxic element (this was only possible for lead and aluminium) or, in the absence of any measured value(s), the reported LOQ and by applying a factor of 5 to allow flexibility with respect to representativeness and homogeneity. An overview is presented in Table E.3.

TABLE E.3 Values selected by the Panel for the calculation of the potential exposure to toxic elements from the use of E 551 based on the available data.

	Lead	Mercury	Arsenic	Aluminium
Current limits in the EU specifications (mg/kg)	5	1	3	
Rounded up highest measured value (mg/kg)	2.5	–	–	450
LOQ multiplied by factor of 5 (mg/kg)		0.25	0.5	

The Panel emphasises that the choice of the factor, as well as other considerations, such as on multiple sources of exposure, to conclude on the maximum limit for toxic elements in the specifications is in the remit of risk management. The numbers used here are merely taken to support the risk assessment of these toxic element as presented below. The outcome of the risk assessment for these two scenarios is illustrated in Table E.4 (all population groups and infants < 16 weeks of age).

TABLE E.4 Risk assessment for toxic elements from the use of silicon dioxide (E 551) for general population.

(i) Considering the presence of toxic elements at the current limits of the EU specifications for E 551 (Commission Regulation (EU) No 231/2012)			
Exposure to E 551 (mg/kg bw per day)	**MOE for Pb at 5 mg/kg**	**% of the TWI for Hg at 1 mg/kg**	**MOE for iAs at 3 mg/kg**
18[a]	5	3	1
50[b]	2	9	0.4
2[c]	50	0.4	10
3[d]	33	0.5	7
0.8[e]	125	0.1	25
1.1[f]	91	0.2	18

(ii) Considering the presence of toxic elements at the rounded up highest measured value, or the reported LOQ by applying a factor of 5				
Exposure to E 551 (mg/kg bw per day)	**MOE for Pb at 2.5 mg/kg**	**% of the TWI for Hg at 0.25 mg/kg**	**MOE for iAs at 0.5 mg/kg**	**% of the TWI for Al at 450 mg/kg**
18[a]	11	0.8	6	6
50[b]	4	2	2	16
2[c]	100	0.1	60	0.6
3[d]	67	0.1	40	0.9
0.8[e]	250	< 0.1	150	0.3
1.1[f]	181	< 0.1	109	0.4

[a]Highest mean exposure among the different population groups (refined non-brand-loyal scenario– children (Table E.1).
[b]Highest 95th percentile exposure among the different population groups (refined non-brand-loyal scenario -children (Table E.1).
[c]Mean consumption to silicon dioxide (E 551) in infant formulae (FC 13.1.1 or FC 13.1.5.1) considering maximum reported level (Table 6).
[d]High consumption to silicon dioxide (E 551) in infant formulae (FC 13.1.1 or FC 13.1.5.1) considering maximum reported level (Table 6).
[e]Highest mean consumption to silicon dioxide (E 551) in infant formulae (FC 13.1.1 or FC 13.1.5.1) considering the mean of reported levels (Table 6).
[f]Highest high consumption to silicon dioxide (E 551) in infant formulae (FC 13.1.1 or FC 13.1.5.1) considering the mean of reported levels (Table 6).

The resulting figures in Table E.4 show that for Pb and Hg, the presence of these toxic elements in E 551 either at the current specifications limit values or at the levels selected by the Panel would not give rise to concern, as well as for Al, considering the rounded up highest measured value. For As, taking into account the use of E 551 for the general population, the calculated MOE values considering the current limit in the EU specification were considered to be insufficient.

The Panel noted that maximum levels for lead and arsenic in infant formula are set by Reg. (EC) No 2023/915. Therefore, the Panel calculated the impact of the level of the toxic elements lead and arsenic in the food additive on infant formulae as placed on the market and compared that with the legal limits for these elements in the final formula for the infants below 16 weeks of age. This results in a fraction of lead and arsenic from the use of the food additive up to 0.3% and 0.1% of maximum limit permitted in the final product (infant formulae).

ANNEXES

Annexes A–H can be found in the online version of this output ('Supporting information' section).

Annex A: Literature searches.
Annex B: Genotoxicity studies assessment by the ccWG Genotoxicity.
Annex C: List of studies with Risk of Bias (RoB) score 3 or 4.
Annex D: Weight of Evidence table.
Annex E: Dietary exposure (food for special medical purpose (FSMP) consumer only scenario for infants above 16 weeks of age and young children (or toddlers)).
Annex F: Summary of in vivo ADME studies included in the assessment.
Annex G: Summary tables of the toxicity studies included in the assessment.
Annex H: Summary of the toxicity studies with RoB score 3 considered as supportive evidence.