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Re-evaluation of thaumatin (E 957) as food additive

EFSA Panel on Food Additives and Flavourings (FAF),
Maged Younes, Gabriele Aquilina, Laurence Castle, Karl-Heinz Engel, Paul Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Rainer Gürtler, Ursula Gundert-Remy, Trine Husøy, Melania Manco, Wim Mennes, Sabina Passamonti, Peter Moldeus, Romina Shah, Ine Waalkens-Berendsen, Detlef Wölfle, Matthew Wright, Monika Batke, Polly Boon, Ellen Bruzell, James Chipman, Riccardo Crebelli, Rex Fitzgerald, Cristina Fortes, Thorhallur Halldorsson, Jean-Charles LeBlanc, Oliver Lindtner, Alicja Mortensen, Evangelia Ntzani, Heather Wallace, Consuelo Civitella, Zsuzsanna Horvath, Federica Lodi, Alexandra Tard and Giorgia Vianello

Abstract

The present opinion deals with the re-evaluation of thaumatin (E 957) when used as a food additive. Thaumatin is a natural plant protein, consisting of thaumatin I and thaumatin II proteins together with minor amounts of plant constituents, obtained by acidic aqueous extraction of the arils of the fruit of *Thaumatococcus daniellii* plant. The Panel followed the conceptual framework for the risk assessment of certain food additives and considered that thaumatin is a digestible protein; adequate exposure estimates were available; there was no concern with respect to the genotoxicity; no conclusion on oral allergenicity could be drawn from the available human data; no adverse effects were observed in sub-chronic toxicity studies in rats and dogs at the highest dose tested of up 5,200 and 1,476 mg/kg bodyweight (bw) per day, respectively, and in a prenatal developmental toxicity study up to 2,000 mg/kg bw per day; moderate confidence in the body of evidence supported the absence of association between exposure to thaumatin and adverse health outcomes. Therefore, the Panel concluded that there is no need for a numerical acceptable daily intake (ADI) for thaumatin (E 957) and, based on a margin of safety (MOS) of 5,417, considered to be an underestimate and derived using the highest 95th percentile (P95) exposure of 0.48 mg/kg bw per day in consumers only, there is no safety concern for thaumatin (E 957) at the regulatory maximum level exposure assessment scenario, which was considered the most appropriate. The Panel recommended that European Commission considers introducing in the EU specifications for thaumatin (E 957) a new specification limit for the minimum combined content of thaumatin I and II proteins in E 957, a specification limit for yeast, mould counts and *Salmonella* spp and lowering the existing maximum limit for arsenic along with the inclusion of maximum limits for mercury and cadmium.

Keywords: thaumatin, E 957, food additive, sweetener

Requestor: European Commission

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Correspondence: fip@efsa.europa.eu

Panel members: Maged Younes, Gabriele Aquilina, Laurence Castle, Karl-Heinz Engel, Paul Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Ursula Gundert-Remy, Rainer Gürtler, Trine Husøy, Melania Manco, Wim Mennes, Peter Moldeus, Sabina Passamonti, Romina Shah, Ine Waalkens-Berendsen, Detlef Wölfle and Matthew Wright.

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Summary

The present opinion deals with the re-evaluation of thaumatin (E 957) when used as a food additive.

Thaumatin (E 957) is authorised as a food additive in the European Union (EU) in accordance with Annex II to Regulation (EC) No 1333/2008 on food additives and its specifications are defined in the Commission Regulation (EU) No 231/2012.

Thaumatin was previously assessed by both the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1986 (JECFA, 1986) and the Scientific Committee on Food (SCF) (1985, 1989). Following these evaluations, thaumatin (E 957) was considered acceptable for use and the acceptable daily intake (ADI) was established as 'not specified'. A more recent opinion on the safety of thaumatin for use as a feed additive concluded that there were no concerns for consumer safety from the use of thaumatin in feed and water for drinking for all species, as 'thaumatin is a highly digestible protein and no residues in edible tissues/products are expected' (EFSA FEEDAP Panel, 2011). In 2015, the former EFSA ANS Panel issued a scientific opinion on a proposed extension of use of thaumatin (E 957) concluding that, based on the existing toxicological evaluations, the proposed extension of uses and changes to use levels would not represent a safety concern (EFSA ANS Panel, 2015).

The current risk assessment was carried out based on structured protocols (EFSA, 2020a,b) on hazard identification and characterisation (EFSA, 2020a) and on exposure assessment (EFSA, 2020b). The protocols defined the strategy to be applied for collecting and selecting data, appraising the relevant evidence and analysing and integrating the evidence.

According to Commission Regulation No 231/2012, thaumatin (E 957) is obtained by acidic aqueous extraction of the arils of the fruit of *Thaumatococcus daniellii* (Benth) plant. This food additive is a natural plant protein material: it consists essentially of the proteins thaumatin I and thaumatin II, together with minor amounts of plant constituents, such as arabinogalactan and arabinoglucuronoxylan polysaccharides, derived from the source material. Based on the data provided by interested business operators (electrophoretic analysis), it was shown that thaumatin (E 957) does not only contain the two thaumatin proteins but also other proteins and/or peptides.

The Panel took note that the current EU specification for the minimum purity assay reflects the total protein content in E 957, i.e. not less than 93% (established using Kjeldahl method with nitrogen conversion factor (NCF) of 6.2), whereas the actual content of thaumatin I and II proteins may be only four-fifths of this total, as indicated by one interested business operator. Therefore, the Panel considered that a new specification limit for the minimum combined content of thaumatin I and II proteins in E 957, determined by a validated analytical methodology for quantifying the thaumatin proteins, e.g. high-performance liquid chromatography (HPLC), should be introduced in the EU specifications for E 957.

Based on the analytical data provided by the interested business operators and the dietary exposure estimation to the food additive, the Panel calculated the potential exposure to the toxic elements from the use of E 957 (Appendix E). The Panel considered appropriate to lower the existing EU maximum limit for arsenic and to add maximum limits for mercury and cadmium to the EU specifications for thaumatin (E 957).

Because of its botanical origin, thaumatin (E 957) may be prone to microbiological contamination. The Panel noted that in addition to the already included EU specification limits for total aerobic microbial count and *E. coli*, further microbiological specifications for yeasts, moulds and *Salmonella* spp should be introduced. In addition to microbiological contamination, mycotoxins and pesticides residues may be possible contaminants in E 957. Based on the data and information provided, the Panel considered that there is no concern with respect to contamination by mycotoxins in E 957 and thus no need to introduce limit values for mycotoxins in the EU specifications of this food additive. Regarding pesticides, no residues were detected in one batch of thaumatin (E 957); therefore, the Panel considered that limit values for pesticides in the EU specifications of E 957 are not needed, as long as it is assured that arils are collected from plants that are not commercially cultivated.

Only data on thaumatin digestibility (*in vitro* and *in vivo*) were received from the interested business operators and no new data were identified in the literature. No other data on absorption, distribution, metabolism and excretion (ADME) were available. Based on the available studies, the Panel considered that thaumatin is a readily digestible protein. Acute toxicity studies in mice and rats showed no adverse effects up to 20 and 21 g/kg body weight (bw).

Thaumatin (E 957) did not show a genotoxic potential in a limited bacterial mutation assay and in a dominant lethal test in mice. The Panel noted that the available data set is not aligned with current

requirements for genotoxic hazard identification. However, based on the nature of E 957, the Panel overall concluded that there is no concern with respect to genotoxicity. The toxicology data set consisted of studies, assessed as relevant and reliable based on the criteria established in the draft protocol on hazard identification and characterisation of sweeteners (EFSA, 2020a), on short-term and sub-chronic toxicity in rats and dogs, prenatal developmental toxicity in rats and sensitisation in different experimental animal species. Human data were also available and consisted of three limited human oral intervention studies and two observational studies (exposure to thaumatin via inhalation). No reproductive, chronic toxicity or carcinogenicity studies were available. In the sub-chronic toxicity studies in rats, no effects were observed on testis, uterus or ovaries.

Overall, the repeated dose toxicity studies and a prenatal developmental toxicity study in animals did not identify any adverse effects. Allergenicity via oral exposure was considered unlikely based on animal studies, but was possible via inhalation based on two observational studies in humans. However, indications of allergenicity of thaumatin (E 957) via inhalation in occupational settings are not considered relevant for dietary exposure.

Thaumatin (E 957) is authorised in the EU in 15 food categories with maximum permitted levels (MPLs) ranging from 0.5 to 400 mg/kg and at *quantum satis* (QS) in the three food categories (FCs) of table-top sweeteners. Thaumatin (E 957) is not authorised according to Annex III of Regulation (EC) No 1333/2008. An interested business operator provided EFSA with one use level only (100 mg/kg) in foods belonging to FC 11.4.3 table-top sweeteners in tablets.

Dietary exposure to the food additive was estimated according to different exposure scenarios based on consumers only. The highest mean and 95th percentile (P95) exposure among consumers of one or more food categories containing thaumatin (E 957) was found in adolescents (0.08 and 0.33 mg/kg bw per day), while among consumers of individual food categories the highest mean and P95 exposure was found in consumers only of FC 05.1 chocolate and chocolate products in children with 0.17 and 0.48 mg/kg bw per day, respectively. The Panel considered that the exposure to thaumatin (E 957) from its use as a food additive was overestimated in the regulatory maximum level exposure assessment scenario, given that exposure calculations based on the MPLs/maximum reported use levels were considered applicable to all foods within each food category, while the percentage of the foods in a subcategory labelled with thaumatin (E 957) in Mintel's Global New Products Database (GNPD) was maximally 3.2%.

In the refined brand-loyal scenario, the highest mean exposure was found in children and adolescents (0.01 mg/kg bw per day) while the highest P95 was 0.02 mg/kg bw per day in adults. Infants and toddlers exposure was not presented for this scenario due to no reported consumption of table-top sweeteners and because of only few consumers (less than 6) found per survey, respectively. For this scenario, the Panel considered that the exposure to thaumatin (E 957) was underestimated due to the overall uncertainties and in particular that use levels were only available for table-top sweeteners, whereas thaumatin (E 957) is labelled on other foods such as flavoured drinks (especially in energy drinks), food supplements as well as in a small number of other subcategories in GNPD.

Based on a rat 13-week no observed adverse effect level (NOAEL) of 5,200 mg/kg bw per day, the highest dose tested in males, including a factor of 2 for extrapolation from sub-chronic to chronic exposure (EFSA Scientific Committee, 2012a), a margin of safety (MOS) of 5,417 was derived using the highest P95 exposure in consumers only (0.48 mg/kg bw per day based on the regulatory maximum level exposure assessment scenario). The Panel considered that the MOS is an underestimation since the exposure data are based on a regulatory maximum level exposure assessment scenario.

According to the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010 (EFSA ANS Panel, 2014) and given that:

- thaumatin is a digestible protein;
- adequate exposure estimates were available;
- there was no concern with respect to the genotoxicity;
- no conclusion on oral allergenicity could be drawn from the available human data;
- no adverse effects were observed in sub-chronic toxicity studies in rats and dogs at the highest dose tested (5,200 mg/kg bw per day and 1,476 mg/kg bw per day, respectively) and in a prenatal developmental toxicity study (2,000 mg/kg bw per day);
- moderate confidence in the body of evidence supported the absence of association between exposure to thaumatin and adverse health outcomes

the Panel concluded that there is no need for a numerical ADI for thaumatin (E 957). Based on a calculated MOS of 5417, considered to be an underestimate, the Panel concluded that there is no

safety concern for thaumatin (E 957) at the regulatory maximum level exposure assessment scenario, which was considered the most appropriate.

The Panel recommends the European Commission to consider:

- introducing the CAS number 53850-34-3 in the EU specifications for E 957
- introducing a new specification limit for the minimum combined content of thaumatin I and II proteins in E 957, determined by using a validated analytical methodology for quantifying specifically the thaumatin proteins, e.g. HPLC;
- lowering the existing EU maximum limit for arsenic and adding maximum limits for mercury and cadmium to the EU specifications for E 957;
- introducing a specification limit for yeast and mould counts and *Salmonella* spp in the EU specifications for E 957, based on the information provided by the interested business operators and Panel considerations.

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1. Introduction

The present opinion deals with the re-evaluation of thaumatin (E 957) when used as a food additive.

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

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

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

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

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

1.1.2. Terms of Reference

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

1.2. Information on existing authorisations and evaluations

Thaumatin (E 957) is authorised as a food additive in the EU in accordance with Annex II to Regulation (EC) No 1333/2008 on food additives and its specifications are defined in the Commission Regulation (EU) No 231/2012[3].

The SCF completed the safety evaluation of thaumatin (E 957) in its initial review of sweeteners in 1984 (SCF, 1985). The history of use of the source material *Thaumatococcus daniellii*, the properties of the two main active ingredients (the proteins thaumatin I and thaumatin II) and the use levels of thaumatin were reviewed. Additionally, the estimated intake of thaumatin, the allergenicity, digestibility, mutagenicity and sub-chronic toxicity of thaumatin were part of the safety evaluation. The SCF expressed some concerns regarding the potential for receptor binding and possible endocrine activity,

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

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

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

and therefore, an acceptable daily intake (ADI) could not be established. Thaumatin was considered to be 'temporarily acceptable' until further information could be evaluated.

The safety of thaumatin was re-addressed in 1989 (SCF, 1989). The Committee reviewed data on relative organ weights and the histopathology of various endocrine organs in rats and dogs, the analysis of structure/receptor relationships, the results of a 90-day study in humans and the results of a 4-week study to investigate thyroid function in rats. Thaumatin was considered acceptable from a toxicological point of view based on the lack of consistent treatment-related effects on relative organ weights and the histopathology of various endocrine organs in two rat studies and a single dog study; no treatment-related changes in three serum parameters in humans exposed to large doses of thaumatin (280 mg/day) for 12 weeks; the structural and conformational considerations that thaumatin is unlikely to give rise on digestion to neuroendocrine or hormonally active peptides; and the small exposure to thaumatin, arising from a few food commodities and estimated to be 1–2 mg/person per day from use at levels up to about 30 ppm. Therefore, thaumatin was considered acceptable for use, and the ADI was established as 'not specified'.

Thaumatin (E 957) was initially reviewed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its 27th meeting; however, neither an appropriate long-term animal study nor adequate studies in humans were available, so no ADI was allocated (JECFA, 1983).

In 1985, JECFA reviewed a comprehensive data set for thaumatin that included digestibility, mutagenicity, teratogenicity, allergenicity, acute and short-term animal toxicity and human studies (JECFA, 1986). Based on the results of these studies, JECFA noted that the digestion of thaumatin would not be different from that of other dietary proteins; hormonally active polypeptides would not be likely to result from the digestion of thaumatin; there was no evidence of mutagenic, teratogenic or allergenic effects; JECFA indicated that no effect levels of 30 and 10 g/kg body weight per day were observed in 90-day studies in rats and dogs, respectively, and no treatment-related changes were observed following the consumption of 280 mg thaumatin/day for 13 weeks by human volunteers. JECFA established that the ADI was 'not specified', based on an anticipated maximum daily intake of 2 mg/person per day, and 'in view of the fact that thaumatin makes an insignificant contribution to the normal protein diet and is metabolised into normal body constituents'. As indicated in 2015 by the ANS Panel in the scientific opinion on a proposed extension of use of thaumatin (E 957), the NOAELs of 30 and 10 g/kg body weight per day from the JECFA evaluation have been mistakenly reported, but should have been reported as 30 and 10 g/kg feed.

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) evaluated the safety of thaumatin for use in all animal species in 2011 (EFSA FEEDAP Panel, 2011). Safety for the target species was based on the lowest no observed adverse effect level (NOAEL) identified by JECFA in 1986 (however, wrongly reported), which was 10 g/kg body weight per day in dogs (the highest dose tested). From this NOAEL, the FEEDAP Panel calculated the maximum safe feed concentrations for the target species (range 1,667–5,000 mg/kg feed) and concluded that the proposed use levels of 1–5 mg thaumatin/kg feed were safe for all animal species and included a 'considerable margin of safety'. Owing to this large margin of safety, it was concluded that thaumatin could be simultaneously administered in feed and water for drinking. The FEEDAP Panel also concluded that there were no concerns for consumer safety from the use of thaumatin in feed and water for drinking for all species, as 'thaumatin is a highly digestible protein and no residues in edible tissues/products are expected'.

The former EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) issued a scientific opinion on a proposed extension of use of thaumatin (E 957) in 2015 (EFSA ANS Panel, 2015). In this opinion, the ANS Panel considered a comment from the applicant reporting that in the NOAELs from the JECFA evaluation, there must have been errors in transcription, since from the description of the studies, the NOAEL appeared to be 3.0% in the diet for both animal species, corresponding to a daily dose of approximately 2,700 mg/kg body weight (bw) per day in rats (2,394–2,500 in male rats; 2,822–2,925 in female rats), and approximately 1,400 mg/kg bw per day in dogs (range 1,101–1,600 mg/kg bw per day in male dogs; 1,249–1,791 mg/kg bw per day in female dogs) (EFSA ANS Panel, 2015). On the basis of this opinion, in 2018, the Regulation (EC) No 1333/2008 was amended to authorise the use of thaumatin (E 957) as a flavour enhancer in products of food categories 12.6 'Sauces' and 15.1 'Potato-, cereal-, flour- or starch-based snacks' at a maximum level of 5 mg/kg in each food category.[4]

[4] Commission Regulation (EU) 2018/677 of 3 May 2018 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the use of thaumatin (E 957) as a flavour enhancer in certain food categories.

The Panel acknowledged the request received by JECFA, at the 52nd session of the Joint FAO/WHO Food Standards Programme Codex Committee on Food Additives (CX/FA 21/52/12, May 2021),[5] as regards thaumatin II sourced from recombinant plant technologies. The request seeks for re-evaluation of E 957 to modify the existing definition to include the new manufacturing process and any relevant specification for the new source of the food additive (i.e. thaumatin II produced recombinantly in green plants).

Thaumatin is also approved for use in the USA, Canada, Australia and New Zealand, Switzerland, Israel, China, Japan, Hong Kong, Korea, Singapore, Mexico, Brazil and South Africa (EFSA ANS Panel, 2015).

2. Data and methodologies

The current risk assessment was carried out by the EFSA Panel on Food Additives and Flavourings (FAF Panel) in the context of Regulation (EC) No 257/2010. Structured protocols on hazard identification and characterisation (EFSA, 2020a) and on exposure assessment (EFSA, 2020b) were developed in line with the principles of the EFSA PROMETHEUS project (PROmoting METHods for Evidence Use in Scientific assessments) (EFSA, 2015a). The protocols define the strategy to be applied for collecting and selecting data, appraising the relevant evidence and analysing and integrating the evidence in order to draw conclusions that will form the basis for the scientific opinions.

2.1. Data

The FAF Panel was not provided with a newly submitted dossier. EFSA launched public calls for data[6,7,8] and contacted interested parties to collect relevant information.

The Panel based its assessment on information submitted to EFSA following the public calls for data, information from previous evaluations and additional available literature up to May 2021.

The steps followed for the acquisition of data and their selection are documented in detail in Appendix A.

Food consumption data used to estimate the dietary exposure to the food additive thaumatin (E 957) were derived from the EFSA Comprehensive European Food Consumption Database (Comprehensive Database[9]).

The Mintel's Global New Products Database (GNPD) was checked to identify the uses of the food additive thaumatin (E 957) in food and beverage products and food supplements. The Mintel's GNPD is an online database that contains the compulsory ingredient information present on the label of numerous products.

2.2. Methodologies

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

The FAF Panel assessed the safety of thaumatin (E 957) as a food additive in line with the principles laid down in Regulation (EU) 257/2010 and in the relevant guidance documents: Guidance on submission for food additive evaluations by the Scientific Committee on Food (SCF, 2001) and the Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012).

In the context of this re-evaluation, the Panel followed the *'Statement on a conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EC) No 257/2010'* (EFSA ANS Panel, 2014).

The draft protocol for the hazard identification and characterisation of sweeteners was published on EFSA's website for comments, and the online public consultation was made available until

[5] http://www.fao.org/fao-who-codexalimentarius/sh-proxy/tr/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-711-52%252F2021SepWD%252Ffa52_12e.pdf

[6] Call for technical and toxicological data on sweeteners authorised as food additives in the EU - Extended deadline for submitting data: 30/6/2018: https://www.efsa.europa.eu/sites/default/files/engage/170621.pdf

[7] Call for technical data on sweeteners authorised as food additives in the EU: http://www.efsa.europa.eu/sites/default/files/consultation/callsfordata/190513-cfd.pdf

[8] Call for food additives usage level and/or concentration data in food and beverages intended for human consumption (Batch 7).

[9] Available online: http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

19 September 2019. A technical report on the outcome of this public consultation with the overview of the comments received and the general responses from EFSA was published (EFSA, 2020a).

In animal studies, when the test substance was administered in the feed or in the drinking water but doses were not explicitly reported by the authors as mg/kg bw per day based on actual feed or water consumption, the daily intake 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, 2012a,b) are applied. In the case of other animal species, the default values used by JECFA (2000) are used. 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'. When in adult human studies (aged above 18 years) the dose of the test substance administered was reported in mg/person per day, the dose in mg/kg bw per day was calculated by the Panel using a body weight of 70 kg as default for the adult population as described in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012a,b).

Dietary exposure to thaumatin (E 957) from its use as a food additive was estimated combining food consumption data available within the EFSA Comprehensive Database with the maximum levels according to Annex II to Regulation (EC) No 1333/2008[10] and/or reported use levels and analytical data submitted to EFSA following a call for data. Different scenarios were used to calculate the exposure (see Section 3.4). Uncertainties in the exposure assessment were identified and discussed.

The draft protocol for assessing dietary exposure to sweeteners was published on EFSA's website for comments, and the online public consultation was made available until 22 November 2019. A technical report on the outcome of this public consultation with the overview of the comments received and the general responses from EFSA was published (EFSA, 2020b).

The methods for hazard identification followed to perform this risk assessment are detailed in Appendix A. In short, following data retrieval and screening for relevance, risk of bias (RoB) was evaluated (studies in Section 3.5.4) and studies classified into Tiers from 1 to 3, corresponding to decreasing levels of internal validity.[11] If Tier 1 and 2 studies were available, Tier 3 studies were not considered further in the assessment. Initial confidence ratings were performed for all studies based on study design for each relevant, reported outcome. Animal and human studies were evaluated separately. The confidence in the evidence for the absence or presence of adverse effects was assessed in a weight of evidence (WoE) approach. For each outcome, the initial confidence rating may have been downgraded based on either a concern for risk of bias after taking all studies into account, an unexplained inconsistency, relevance of endpoints (in term of adversity) and/or imprecision (including statistical power); it may have been upgraded for a large magnitude of effect, evidence for a dose-response, consideration of residual confounding (human studies only) and consistency across study designs and experimental model systems. Finally, the level of evidence for (no) health effect was assessed, as outlined in step 1.14 of the draft protocol for the assessment of hazard identification and characterisation of sweeteners (EFSA, 2020a) and in the US National Toxicology Program (NTP) Handbook for conducting a literature-based health assessment using the OHAT approach for systematic review and evidence integration (NTP-OHAT, 2019) with some modifications (Appendix A).

3. Assessment

3.1. Technical data

3.1.1. Identity of the substance

According to Commission Regulation No 231/2012, thaumatin (E 957) is obtained by acidic aqueous extraction of the arils of the fruit of *Thaumatococcus daniellii* (Benth) plant. This food additive is a natural plant protein material: it consists essentially of the proteins thaumatin I and thaumatin II together with minor amounts of plant constituents.

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

[11] 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.

In response to the EFSA calls for data, interested business operators stated that thaumatin (E 957) is as an odourless powder, made mainly of proteins thaumatin I and thaumatin II, obtained by water extraction from the arils of the fruits of *T. daniellii*. This plant is a perennial herb of the Marantaceae family which grows wild in West and Central Africa. According to the interested business operators, the plant is not commercially cultivated. Detailed information on the taxonomy of the plant as well as on the growth and harvesting conditions of the plant was provided by interested business operators (Documentation provided to EFSA nr: 1, 2, 4, 5) and it is also available in the scientific literature (Waliszewski et al., 2005, 2012). The Panel noted that *T. daniellii* is not included in the EFSA Compendium of Botanicals[12] which lists botanicals that are reported to contain naturally occurring substances of possible concern for human health when present in food.

Naturally occurring thaumatin consists of six closely related proteins (I, II, III, a, b and c), all with a molecular mass of 22 kDa (207 amino acids) (Van der Wel and Loeve, 1972; Ledeboer et al., 1984). The predominant proteins in the food additive E 957 are thaumatin I and thaumatin II present in a ratio 2:1, each cross-linked by eight disulfide bridges. The amino acid sequences of Thaumatin I and Thaumatin II differ by only two residues. The molecular weights of the two proteins are 22,209 and 22,293 g/mol for thaumatin I and thaumatin II, respectively. The complete amino acid sequences of Thaumatin I and II and their tertiary structure were originally described by Iyengar et al. (1979) and Edens et al. (1982). One interested business operator provided adapted information from these two references, i.e. reporting the asparagine and glutamine residues present in thaumatin I and II as such (asparagine and glutamine amino acids) and not as their deamination products (aspartic and glutamic acids) incorrectly indicated in the two references (Documentation provided to EFSA nr: 4).[13] The three-dimensional structures of thaumatin I and thaumatin II have been studied and described in the scientific literature (De Vos et al., 1985; Masuda et al., 2011).

A sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in one batch of E 957 in order to separate the proteins contained in the sample according to their molecular weight and estimate their relative proportions (Documentation provided to EFSA nr: 3). This showed that thaumatin (E 957) does not only contain the two thaumatin proteins but also other proteins, as indicated by minor bands observed at 26 and 38 kDa and by diffuse bands present below 15 kDa. Calculation of the relative proportions of the bands indicated that the thaumatin I and II proteins account for 81% of total proteins, with approximately 6% of the total represented by other proteins of higher molecular weight and 14% represented by other proteins and/or peptides of lower molecular weight.

The non-proteinaceous fraction present in E 957 consists of carbohydrates and ash (e.g. inorganic salts). The carbohydrates are co-extracted polysaccharidic impurities, such as arabinogalactans and arabinoglucuronoxylans. They are normal constituents of the plant cell walls and the surrounding mucilage gel material of the arils. Most of them are removed during thaumatin purification by precipitation and ultrafiltration and their content ranges from 0% to 2% (Documentation provided to EFSA: 1, 2, 4, 5).

The Panel noted that thaumatin (E 957) may be traded under the commercial name Talin®.

3.1.2. Specifications

The specifications for the food additive thaumatin (E 957), as defined in the Commission Regulation (EU) No 231/2012 and by JECFA (2006), are listed in Table 1.

[12] Available online: https://www.efsa.europa.eu/it/data/compendium-botanicals

[13] Full information on amino acid sequence, secondary and tertiary structure of thaumatin I and thaumatin II available at: https://www.uniprot.org/uniprot/P02883.

Table 1: Specifications for thaumatin (E 957) according to Commission Regulation (EU) No 231/2012 and JECFA (2006)

	Commission Regulation (EU) No 231/2012	**JECFA (2006)**
Synonyms		INS No. 957
Definition		Obtained by aqueous extraction (pH 2.5–4.0) of the arils of the fruit of *Thaumatococcus daniellii* (Benth); consists essentially of the proteins thaumatin I and thaumatin II together with minor amounts of plant constituents derived from the source material.
CAS Nr.		53850-34-3
Einecs	258-822-2	
Chemical name	Thaumatin is obtained by aqueous extraction (pH 2.5–4) of the arils of the fruit of strains of *Thaumatococcus daniellii* (Benth) and consists essentially of the proteins thaumatin I and thaumatin II together with minor amounts of plant constituents derived from the source material.	
Chemical formula	Polypeptide of 207 amino acids	
Molecular weight	Thaumatin I 22209 Thaumatin II 22293	Thaumatin I 22209 Thaumatin II 22293
Assay	Not less than 15.1% nitrogen on the dried basis equivalent to not less than 93% proteins (N × 6,2)[(a)]	Not less than 15.1% nitrogen on the dried basis equivalent to not less than 93% protein (N × 6.2)
Description	Odourless, cream-coloured powder. Approximately 2,000–3,000 times as sweet as sucrose	Odourless, cream-coloured powder
Identification		
Solubility	Very soluble in water, insoluble in acetone	Very soluble in water, insoluble in acetone
Ninhydrin test		To 5 mL of a 1 in 1,000 mL solution of the sample, add 1 mL of freshly prepared triketohydrine hydrate (ninhydrin) solution (dissolve 200 mg of triketohydrine hydrate in water and dilute to 100 mL). A bluish colour is produced.
Infrared absorption		The infrared spectrum of a potassium bromide dispersion of the sample (1–2 mg of sample ground in a mortar with 100–200 mg potassium bromide) corresponds to the infrared spectrum below.[(b)] Characteristic maxima of absorption are shown at the following wavenumbers: 3300, 2960, 1650, 1529, 1452, 1395, 1237, 1103 and 612 cm^{-1}.
Purity		
Loss on drying	Not more than 9% (105°C to constant weight)	Not more than 9.0% (105°C to constant weight).
Carbohydrates	Not more than 3% (expressed on dry weight basis)	Not more than 3.0% on the dried basis.
Sulfated ash	Not more than 2% (expressed on dry weight basis)	Not more than 2.0% on the dried basis
Aluminium	Not more than 100 mg/kg (expressed on dry weight basis)	Not more than 100 mg/kg. Determine by atomic absorption spectroscopy.

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Arsenic	Not more than 3 mg/kg (expressed on dry weight basis)	
Lead	Not more than 3 mg/kg (expressed on dry weight basis)	Not more than 3 mg/kg. Determine using an atomic absorption technique appropriate to the specified level.
Spectrophotometry		The specific absorption, $A^{1\%}$ 1 cm at the wavelength of maximum absorption (about 279 nm) shall be not less than 11.5 and not more than 13.0 determined on the dried basis and using a 1 in 100 w/v solution of the sample in water at pH 2.7.
Microbiological criteria		
Total aerobic microbial count	Not more than 1,000 colonies per gram	Total aerobic plate count: Not more than 1,000 cfu/g
Escherichia coli	Absent in 1 g	Negative in 1 g

(a): According to JECFA (2006) specifications, it is recommended to proceed as direct nitrogen determination (Kjeldahl Method; Volume 4), Method II.
(b): Available online: http://www.fao.org/fileadmin/user_upload/jecfa_additives/docs/Monograph1/Additive-462.pdf

The Panel observed that the information currently reported under the chemical name would be more appropriate for the definition of thaumatin (E 957). In addition, the Panel also noticed that the term '*strains*' currently reported to describe E 957 is not appropriate to refer to botanical species and it can be omitted as in line with the JECFA definition.

The Panel noted that the CAS number (53850-34-3) for thaumatin should be included in the current EU specifications for E 957.

Following EFSA calls for data, two interested business operators submitted data and information to support the re-evaluation of thaumatin (E 957). The analytical results provided by the interested business operators on commercial samples of thaumatin (E 957) fulfilled the provisions of the EU specifications for E 957, as laid down in Commission Regulation (EU) No 231/2012 (Documentation provided to EFSA nr: 1, 2, 3, 4, 5, 6).

According to the information provided by the interested business operators, thaumatin (E 957) is commercially extracted with a purity of not less than 94% and it can be further purified (by ultrafiltration) above 97% (Documentation provided to EFSA nr: 1, 2, 4, 5). The Panel observed that the methodology currently indicated in the EU specification for the determination of the minimum purity assay for E 957, i.e. not less than 93% proteins, is based on the nitrogen content and then converted to percentage of the total proteins using a nitrogen conversion factor (NCF) of 6.2 (Kjeldahl method). Upon request of clarification regarding the appropriateness of the use of such NCF, one interested business operator performed analyses of protein purity by reversed phase HPLC (RP-HPLC) and of individual amino acids on five commercial batches of thaumatin (E 957) and compared such analyses to the measured nitrogen in each batch (by Dumas method) with NCF equal to 6.2 (Documentation provided to EFSA nr: 6). The business operator indicated that the resulting data demonstrated good agreement of assessed protein content whether assessed as total proteins, via analysis of individual amino acids or through assessment of total nitrogen and application of the default NCF of 6.2 as set out in the regulation. The Panel noted that in each case, the value obtained for proteins content was always greater than the minimum value of not less than 93% in the EU specifications, i.e. ranging from 99.5% to 99.8% (by RP-HPLC) and from 96.8% to 99.32% (calculated from nitrogen with NCF = 6.2). The average NCF, when based on the sum of amino acids, ranged from 6.17 to 6.38, with a mean of 6.26. With regard to analysis for thaumatin proteins by RP-HPLC, the Panel noted that results are expressed by normalisation of the thaumatin peak against other peaks present in the samples detected at 280 nm, and that this is not a suitable method for assessing the purity of the food additive. It assumes a single extinction coefficient whereas thaumatin, and the potential impurities, may have different content of (UV-absorbing) amino acids with aromatic rings. The Panel also pointed out that it is unclear how the quantification of the individual amino acids was performed, since no details of the method(s) of analysis applied were provided by the interested business operator. Moreover, the Panel noticed that in the amino acid composition provided asparagine

and glutamine were reported as their deamination products, namely aspartic and glutamic acid. Since in the primary amino acids sequence of thaumatin I and thaumatin II, there are 10 and 8 asparagine residues and four and five glutamine residues, respectively, by reporting such amino acids as aspartic and glutamic acid the consequence is that a number of nitrogen are not accounted for. This is because the deaminated products, aspartic and glutamic acid, have only one nitrogen whereas the asparagine and glutamine each have two nitrogen atoms, and thus, this can have implications when calculating an NCF.

The other interested business operator indicated that, considering the molecular weights and amino acid composition of thaumatin I and II proteins, an NCF of 5.8 rather than 6.2 would be appropriate if the proteins in E 957 were solely thaumatin I and thaumatin II. The results of SDS-PAGE analysis in one commercial batch of E 957 in duplicate showed that the food additive was not only comprised of the two thaumatin proteins I and II but also of other proteins, as indicated by minor bands observed at 26 and 38 kDa and by diffuse bands present below 15 kDa, which may represent small protein subunits or peptides. Calculation of the relative proportions of the bands indicated that the thaumatin proteins I and II account for 81% of total protein content, with approximately 6% of the total represented by other proteins of higher molecular weight and 14% represented by other proteins and/or peptides of lower molecular weight (Documentation provided to EFSA nr: 3). Therefore, according to the interested business operator, on the basis that the nitrogen conversion factor of 5.8 would reflect only the nitrogen composition of the thaumatin I and II proteins and not the other proteins present in E 957 and that the Kjeldahl method does not differentiate between different proteins, the use of the general NCF of 6.2 (rounded from 6.25, (FAO, 2003)) is considered appropriate. The total protein content determined by using an NCF of 6.2 in eight batches of E 957 ranged from 94.5% to 97.9%. Moreover, the same interested business operator identified alternative methods for quantifying total proteins in E 957, such as high-performance liquid chromatography with ultraviolet detection (HPLC-UV), HPLC with fluorescence detection (HPLC-FLD) and enzyme-linked immunosorbent assay (ELISA) tests. Nevertheless, the interested business operator stated that such alternative methods do not provide any advantage in terms of reducing the uncertainty of the protein estimates since they can be hardly validated. This owing to the difficulty to retrieve on the market an analytical standard for thaumatin (E 957) with a purity sufficiently defined and consistent to be used as an analytical (calibration) standard. Therefore, the use of the Kjeldahl method with NCF of 6.2 is considered the most appropriate method for calculating the total protein content in E 957 (Documentation provided to EFSA nr: 3).

Based on the above data and information, the Panel noted that the definition of E 957 (currently reported under the chemical name in the EU specifications for E 957) should be amended in order to capture also the other proteins/peptides possibly present in the food additive in addition to thaumatin I and II. A more precise definition of E 957 can be '*Thaumatin is obtained by aqueous extraction (pH 2.5–4) of the arils of the fruit of Thaumatococcus daniellii (Benth) and consists essentially of the proteins thaumatin I and thaumatin II, together with other proteins and/or peptides, and minor amounts of plant constituents derived from the source material*'. The Panel also took note that the reported minimum purity assay reflects the total protein content in thaumatin (E 957), i.e. not less than 94% (established using Kjeldahl method with the current NCF of 6.2), whereas the actual content of thaumatin I and II proteins may be only four-fifths of this total, as indicated by one interested business operator. Therefore, the Panel observed that a new specification limit for the minimum combined content of thaumatin I and II proteins in E 957, determined by a validated analytical methodology for quantifying the thaumatin proteins, e.g. HPLC, should be introduced in the EU specifications for E 957.

With regard to the non-proteinaceous fraction present in the E 957, it consists of carbohydrates and ashes (e.g. inorganic salts) (Documentation provided to EFSA nr: 1, 2, 4, 5).

One interested business operator provided analytical results on the content of total carbohydrates in eight commercial batches of E 957 which ranged from 0.90% up to 1.40% (Documentation provided to EFSA nr: 2). Following clarifications request on the method(s) of analysis applied for the determination of the carbohydrates fraction, one interested business operator pointed out that the direct analytical determination of these polysaccharidic impurities (i.e. arabinogalactan and arabinoglucuronoxylan polysaccharides) is not feasible, as a prior hydrolysis of the sugars is needed. A sample of thaumatin (E 957) was analysed by ion chromatography following hydrolysis with hydrochloric acid and water. The carbohydrate components of the hydrolysed E 957 sample resulted to be xylose/mannose, galactose, arabinose, glucose and rhamnose which comprised 36.19%, 33.58%, 25.62%, 4.00% and 0.61% relative area, respectively (Documentation provided to EFSA nr: 2). The other interested business operator provided data on the sugars profile of five commercial batches of

E 957 (the same batches analysed for the total protein by RP-HPLC and individual amino acids) hydrolysed with hydrochloric acid and then analysed by anion exchange HPLC according to ISO method 11292:1995; and of 26 commercial batches of E 957 analysed by HPLC with refractive index (RI) detection. For both methods, full details were provided. The sugars determined were arabinose, glucose, mannose, xylose, sucrose, galactose, fructose, lactose and maltose. The total carbohydrates content in the five batches analysed by anion exchange HPLC ranged from 0.96% up to 1.57% and in the 26 batches analysed by HPLC with RI detection from 0.10% up to 0.54% (Documentation provided to EFSA nr: 6).

The Panel noted that although different analytical methods can be used, such results on the carbohydrates content in E 957 are broadly below the current EU specification of *'not more than 3%'*. The Panel also noted that similar information, with respect to the carbohydrates profile in thaumatin (E 957), is available in published literature as retrieved by an interested business operator (Documentation provided to EFSA nr: 4).

Regarding the ashes fraction, one interested business operator indicated that minerals sourced from the aril raw materials, water, mineral acids and alkali used in the manufacturing of thaumatin are removed by ultrafiltration down to levels of 0.1% (Documentation provided to EFSA nr: 4, 5). Total ashes levels were measured via gravimetric method (modified ISO method 936:1998) for 35 samples of E 957 and they ranged from 0.02 to 1.32 g/100 g, with a mean value of 0.2 g/100 g (Documentation provided to EFSA nr: 6). The other interested business operator indicated that amounts < 1% (on a dry weight basis) of sulfated ash are present in E 957. The total ash levels in eight batches of E 957 ranged from 0.50% to 0.80% (Documentation provided to EFSA nr: 2, 3).

Concerning the possible presence of uncharacterised material, one interested business operator reported that when five batches of E 957 were analysed for ash, carbohydrate and protein (using a NCF of 6.2), the mass balance for the sum of these three parameters was in the range of 98.33–100.97% (Documentation provided to EFSA nr: 6). Similarly, the other interested business operator indicated that the mass balance of eight batches of E 957 calculated as the sum of the total protein content (using NCF of 6.2), sulfated ash and carbohydrates ranged from 96.2% to 99.77% (Documentation provided to EFSA nr: 3). For both interested business operators, such variations can be justified by the combined measurement uncertainty/analytical error in the three distinct analytical methods used to assess the composition of thaumatin (E 957) and also by the variability in the arils composition (Ojo et al., 2017). Overall, based on such data on the chemical composition of E 957, the Panel considered that no material other than proteinaceous material, carbohydrates and ashes would be expected to occur in the food additive.

With regard to toxic elements, the interested business operators provided analytical data on the levels of lead (Pb), arsenic (As), mercury (Hg), cadmium (Cd) and aluminium (Al) in commercial batches of thaumatin (E 957). Details of the analytical data provided are available in Appendix E (Documentation provided to EFSA nr: 2, 3, 5, 6). Given the differences in the detection limits between the data sets, the results from the two interested business operators were considered consistent for the elements except for Al. The Panel observed a significant inter-batch variability in the detected levels of Al; i.e. Al < 0.5 mg/kg (the limit of quantification (LOQ)) up to 17–35 mg/kg. No information was provided that could explain such a large difference between the various batches in their Al levels. The Panel calculated the potential exposure to the toxic elements from the use of E 957 assuming contamination of the food additive may be up to three times the highest reported level (or LOQ values) for the analysed batches (to account for representativeness, homogeneity and analytical measurement uncertainty) or up to the existing maximum limits for toxic elements and then by calculation pro-rata to the estimates of exposure to the food additive itself (regulatory maximum level and refined brand-loyal exposure assessment scenario; see Section 3.4.1). The exposure calculations to the toxic elements are presented and discussed in Appendix E. The resulting figures showed that the potential exposure to Pb and Al from the uses and use levels of E 957 would not be of concern using both the limit values calculated by the Panel as possible EU specification values (Table E.1, Appendix E) and the existing EU specifications (Table E.2, Appendix E). For As, its margin of safety (MOS)/margin of exposure (MOE) values indicate that a lowering of the existing limit value of 3 mg/kg is recommended, since the reference point (a $BMDL_{01}$) is based on carcinogenicity for which the MOS/MOE should be at least 10,000 (EFSA, 2005; EFSA Scientific Committee, 2012b) and the calculated MOS/MOE can be lower than this value (between 303 and 8, 080, see Table E.2 Appendix E) even though the Panel noted that the BMDL for As (EFSA CONTAM Panel, 2009) is derived from human epidemiological studies and an interspecies extrapolation factor may not be needed. For Cd and Hg, for which no maximum limits are set in the EU specifications, the estimates of exposure are only a

small fraction of their tolerable weekly intake (TWI) values. The Panel noted that interested food business operators indicated that the arils, from which thaumatin (E 957) is extracted, are not commercially cultivated but are harvested from the wild (Section 3.1.3). So thaumatin (E 957) could be susceptible to contamination by toxic elements from environmental sources. Consequently, the Panel considered it appropriate to add limit values for Hg and Cd to the EU specifications alongside limit values for the three elements currently specified, namely Pb, As and Al (Table 1). The Panel noted that the choice of maximum limits for toxic elements in the EU specifications is in the remit of risk management.

Because of the botanical origin, thaumatin (E 957) can be a substrate prone to microbiological contamination. Microbiological data were provided by one interested business operator on 38 thaumatin commercial production batches produced from 2018 to date and the results were given as '*TVC, Staph, Yeast, Mould, and E. coli per gram*'[14] (Documentation provided to EFSA nr: 4). Details on the applied methods were not given except that the analyses were performed by an accredited laboratory. With the exception of four batches, the total viable count (TVC) was < 10/g. One sample had a TVC at 20/g, two samples at 30/g and one sample 420/g. The latter was marked as '*denotes prior to rework or retes*t'. All results for '*Staph'* were < 20/g. For '*Yeast*', one result was reported as 20/g and one sample as 620/g (the same sample that showed the high TVC). Results for '*Mould'* in all 38 samples were < 20/g. For '*E coli*' all results were reported as ND. The same interested business operator also reported results on an extended microbiological monitoring on 18 thaumatin (E 957) commercial production batches from 2017 to 2018, without giving further details on the analysis except the information that the analyses were performed in an accredited laboratory with accredited methods. Results for *Salmonella*, *Listeria* (not further specified by the interested business operator), *E. coli* and *Staph. aureus* are all reported as '*ND per gram'*. The results for *Bacillus cereus* were < 20/g, and for both *Clostridium perfringens* and coliforms were < 10/g. The second interested business operator submitted microbiological data for total aerobic plate count, yeasts, moulds, *E. coli, Staphylococcus aureus*, lactic acid bacteria, *Pseudomonas*, *Salmonella* (the latter two not further specified by the interested business operator) and bile-tolerant negative bacteria in eight thaumatin (E 957) batches (Documentation provided to EFSA nr: 1). In one batch, the total aerobic plate count was 10 colony forming units (cfu) per gram, in all other batches < 10/g. One batch contained 20 and the other seven batches < 20 yeast counts (cfu/g). For moulds, one batch was reported as 40 cfu/g and the remainder as < 20 cfu/g. All results for *E. coli* and *Staphylococcus aureus* were given as '*not detected per gram'*. For *Salmonella*, the results were reported as '*not detected per 25 g'*. All results for lactic acid bacteria were < 10 cfu/g, and for bile-tolerant gram-negative bacteria < 100 cfu/g. Two results for *Pseudomonas* were given as '*absent'* per gram and the other results as < 20/g.

The Panel noted that in addition to the already included EU specification limits for total aerobic microbial count and *E. coli*, further microbiological specifications, including yeasts, moulds and *Salmonella* spp, should be set on the basis of the information provided by the interested business operators.

Because of the botanical origin of the food additive, mycotoxins might be possible contaminants occurring in E 957. Regarding mycotoxins, one business operator indicated the raw materials are tested to ensure that the following maximum limits are respected: aflatoxin B1 < 5 μg/kg, aflatoxins (B1 + B2 + G1 + G2) < 10 μg/kg, ochratoxin A < 15 μg/kg (Documentation provided to EFSA nr: 1). They also provided data for five batches of E 957, i.e. aflatoxins B1, B2, G1 and G2 < 0.1 μg/kg (0.1 being the LOQ), the sum of all positive aflatoxins < 0.4 μg/kg (being 0.4 the LOQ), and ochratoxin A < 0.5 μg/kg (0.5 being the LOQ) (Documentation provided to EFSA nr: 3). According to the other business operator, it is unlikely that mycotoxins would form on the *Thaumatococcus daniellii* raw material and if they were present, being low molecular weight compounds, they would be removed by the ultrafiltration process that used a membrane of > 10 kDa (Documentation provided to EFSA nr: 5). Upon clarification request, they provided an expanded rationale for this conclusion but no analytical data were provided (Documentation provided to EFSA nr: 6). Overall, in view of the above, the Panel considered that there is no concern with respect to a potential contamination by mycotoxins in E 957 and no need to introduce limit values for mycotoxins in the EU specifications of E 957.

Regarding pesticide residues, one interested business operator provided the analysis of one batch of thaumatin (E 957) for the presence of pesticides and no residues were detected (Documentation provided to EFSA nr: 1). Both interested business operators indicated that, despite the botanical origin of the food additive, the risk of pesticide residues is low as the plant *T. daniellii* grows wild in the rainforest. Moreover, the incidental contamination by pesticides from neighbouring cultivations is

[14] The Panel assumed that 'TVC' stands for 'total viable count' and 'Staph' for *Staphylococcus aureus.*

unlikely to occur (Documentation provided to EFSA nr: 2, 5). The Panel saw no need to recommend limit values for pesticides in the specifications of E 957, as long as it is assured that arils are collected from plants that are not commercially cultivated. Nevertheless, the Panel noted that the plant *T. daniellii* could be cultivated (Waliszewski et al., 2012; Khairlani et al., 2020). Should the food additive be prepared in full or in part from cultivated *T. daniellii*, limits for pesticides should be considered for inclusion in the EU specifications in the view of the Panel.

3.1.3. Manufacturing process

Detailed information on the manufacturing process used to produce thaumatin by water extraction from the arils of the fruit of the West African plant *Thaumatococcus daniellii*, including information on the raw materials and the processing aids used, have been provided by the interested business operators and found adequate by the Panel (Documentation provided to EFSA nr: 1, 3, 4, 6). Both interested business operators indicated that all materials used in the production of the membranes employed in the purification steps of E 957 comply with European Regulation (EC) 1935/2004[15] and Commission Regulation (EU) No 10/2011[16].

The Panel noted that the manufacturing of thaumatin proteins through biotechnological procedures, i.e. recombinant DNA technology used for the production of thaumatin proteins in a host organism, is extensively reported and discussed in the peer-reviewed literature (e.g. Hahm and Batt, 1990; Moralejo et al., 1999; Faus, 2000; Healey et al., 2017; Joseph et al., 2019; Masuda et al., 2010). However, the Panel noted that the biotechnological production of thaumatin (E 957) is not mentioned in the EU specifications for E 957, as listed in Commission Regulation (EU) No 231/2012. No interested business operator has indicated the use of such types of synthesis (production method) which may require an assessment according to Regulation (EC) No 1829/2003.

3.1.4. Solubility and particle size

Following EFSA call for technical data, two interested business operators submitted information on solubility and particle size of thaumatin (E 957) (Documentation provided to EFSA nr: 2, 3, 5, 6).

Solubility

One interested business operator performed a visual assessment of the water solubility of E 957 (Documentation provided to EFSA nr: 3). It indicated that due to the expected high water solubility of E 957 (above 200 g/L), as reported in the literature, the chemical analysis method for water solubility as per OECD TG 105[17] can be waived and thus a formal visual assessment was performed. Six replicates of thaumatin (E 957) samples (0.1 g) were mixed with water in the range of 0.1–0.4 mL at room temperature. The mixtures were shaken and stirred by vortex, and after 2 min of standing, they were visually checked for any undissolved parts of the test item. Then, the samples were checked with a laser beam and no particles were detected. Based on the results of the previous assay, the water solubility was determined in the range of 200–1,000 g/L. The approximate water solubility was determined based on the results and the concentrations at which the test item appeared to be fully dissolved. The water solubility of test item at 20 $\pm$ 0.5°C can be considered as less than 500 g/L, but at least approximately 330 g/L, based on the performed visual assessment. The Panel noted that the three analysed batches of E 957 had a purity not compliant with the current EU specifications, i.e. total proteins < 93%.

The other interested business operator determined water solubility in samples of five batches of thaumatin (E 957) according to the OECD TG 105 (Documentation provided to EFSA nr: 6). A preliminary test was carried out with increasing volumes of water added to 100 mg of each sample until fully dissolved. The amount of water required to solubilise the samples was 0.5 mL. Therefore, the solubility based on this test was in the range of 200–1,000 g/L for all the samples. Thaumatin

[15] Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. OJ L 338, 13.11.2004, p. 4–17. Available online: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32004R1935 (Consolidated Version: 07/08/2009).

[16] Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. OJ L 12, 15.1.2011, p. 1–89. Available online: https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32011R0010 (Consolidated version: 23/9/2020).

[17] https://www.oecd-ilibrary.org/docserver/9789264069589-en.pdf?expires=1632232587&id=id&accname=guest&checksum=3228049F2FB7C731A6839CA38EAFA031

solutions of each sample were prepared in triplicate at a concentration of 450 g/L, and constantly shaken for 24 h, 48 h and 72 h, respectively, at 30°C and then left at room temperature for 24 h. The samples were analysed for thaumatin content by HPLC with UV detection at 280 nm. For one of the batches, the concentration of thaumatin determined in the 72 h sample was 18% lower than in the 24 h sample. The difference between the 24 h and 48 h samples was 15% and thus in compliance with the criterion for the validity of the analysis according to OECD TG 105. Therefore, only these results were used to calculate the average saturation mass concentration for that batch. The concentrations of thaumatin determined for the other four batches did also not differ by more than 15%, and the average solubility of thaumatin was calculated to range from 221 to 306 g/L.

The isoelectric point of thaumatin was determined by isoelectric focussing in 7% polyacrylamide gels indicating a pI $\geq$ 11.7, being further confirmed by the precipitation at pH 12.0 of all material in a thaumatin protein solution at 0.1% (van der Wel and Loeve, 1972). Thus, aggregation and precipitation of the thaumatin molecules occur at this pH. However, the aggregation of thaumatin molecules can also occur at different pHs under thermal treatment, e.g. aggregation of thaumatin (E 957) solutions upon heating at 60°C for 15 min and 70°C for 15 min at pH 7 (Kaneko and Kitabatake, 1999). Thaumatin (E 957) was shown to be more thermoresistant under acidic conditions than at neutral or alkaline, thus a pH dependence of thaumatin (E 957) precipitation was observed. The thaumatin proteins at pH $<$ 4 did not form aggregates when heated at 80°C even at prolonged treatments (Kaneko and Kitabatake, 2001).

The Panel noted that the pH of foods where the use of thaumatin (E 957) is authorised would be within the ranges of pH below 7, where thermal precipitation should not occur.

Particle size

One interested business operator assessed particle size of dried thaumatin (E 957) using scanning electron microscopy (SEM) and also studied the volume specific surface area (VSSA) of three samples of E 957 to determine if the food additive would be classified as a nanomaterial (Documentation provided to EFSA nr: 2, 3). The size of the observed particles by SEM ranged between 0.15 and 430 μm. Based on the data measured, the interested business operator concluded that the three analysed samples of E 957 did not contain particles below 100 nm in any one dimension. The Panel noted some shortcomings in this SEM measurement, e.g. non optimal choice of magnification to observe particles in the nanoscale, only the longest dimension of the particles was presented while in the regulatory context, the shortest dimension at least should have been provided. With regard to VSSA analysis, the Panel observed that this analysis cannot be used to ascertain the presence of particles in the nano range (Rauscher et al., 2019).

The other business operator analysed five samples of E 957 by SEM (Documentation provided to EFSA nr: 6). All samples were found to comprise large, flake-like particles up to 400 μm in their longest dimension with some smooth folds to which small particles were attached. No such submicron particles were observed that were not adhered to the surface of the larger particles. The size of these adhered small particles was measured, and the minimum particle size measured was 0.05 μm with the majority of fines smaller than 1 μm. In addition, number-based particle size distributions have been established for each sample and reported. The results showed that the size of all particles sitting on the large flat particles was comparable between all five samples and that the median of this population varied between 0.13 and 0.17 μm with 10th and 90th percentiles at 0.06 μm and 0.48–0.75 μm, respectively. In addition, the particle size distribution of the subpopulation of particles smaller than 0.5 μm was determined. Also, the 10th percentile of this population was 0.06 μm with medians and 90th percentiles ranging between 0.11 and 0.13 μm and 0.31–0.37 μm, respectively. The data provider emphasised that none of these small particles were *'free'* as evidenced by clear backgrounds on the SEM images and reiterates that all measurements were undertaken on small particles adhered to the surface of very large particles.

Overall, the Panel considered that at the specified water solubility of E 957 (221–330 g/L), the food additive is expected to be fully solubilised either in the food matrix or in the gastrointestinal tract, even up to the highest permitted use levels. Therefore, consumers will not be exposed to the material in particle form and the conventional risk assessment according to the EFSA Guidance on food additive (2012) can be followed.

3.1.5. Methods of analysis in food

One interested business operator developed analytical methods based on HPLC for the determination of thaumatin (E 957) in food and beverages (Documentation provided to EFSA nr: 1). RP-HPLC analysis with UV detection (279 nm) is applied in beverages and powder extracts, whereas LC tandem mass spectrometry (LC-MS/MS) is used for food and botanical extracts. In order to improve the sensitivity of the method, prior to LC-MS/MS analysis, the samples are treated with trypsin to hydrolyse thaumatin proteins into a specific common fragment from thaumatin I and II which is then quantified by LC-MS/MS. For milk-containing products, precipitation of caseins before the analysis is also needed. The declared LOQ of this method is 0.1 mg/kg. The Panel noted that this proposed method of analysis was already submitted to EFSA to support the safety evaluation of the extension of use of thaumatin (EFSA ANS Panel, 2015).

The other interested business operator provided references to immunoassays available for the detection and quantitation of thaumatin in food and feedstuffs, which showed good sensitivity and selectivity (e.g. sensitivity ranging from 2 to 100 ng/mL, EFSA FEEDAP Panel, 2011), (Documentation provided to EFSA nr: 4). This interested business operator also indicated the use of UV absorption (in alkaline conditions), gel permeation and ion exchange chromatography as possible techniques to distinguish thaumatin (E 957) from other common proteins (identity tests). These methods are to be applied, preferably, in low protein foods; in proteins-based food (e.g. soy sauce), they are virtually useless. In the open scientific literature other immunoassays for detection and quantification of thaumatin (E 957) are described, reporting low detection limits (e.g. 5 ng/mL, Bodani et al., 1993).

Other analytical techniques exploited for thaumatin (E 957) analysis are flow injection analysis combined with pulsed amperometry using antibody-containing sensors (Sadik et al., 1994) and capillary electrophoresis (Vespalcova and Gregorova, 2003). However, the Panel noted that in all cases, performance criteria for the detection or quantification of thaumatin in food matrices have not been provided.

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

One interested business operator tested long-term (36 months) stability of thaumatin (E 957) at room temperature (20°C) exposed to light, and at temperatures in the range of 7–35°C, at a relative humidity 30–88% in the dark and under accelerated storage conditions in the dark (40 ± 1°C) at a relative humidity of 75% during 6 months, and its stability when used in the proposed food uses, i.e. soft drink formulations (liquid and powdered) and in jellied sweets during 8 months (Documentation provided to EFSA nr: 1).

The Panel noted that these stability studies were already submitted to EFSA to support the safety evaluation of the extension of use of thaumatin (EFSA ANS Panel, 2015) and described as follows by EFSA ANS Panel: *'After 3 years of storage of the additive at room temperature, the total bacterial count was decreased compared to the analysis conducted following production. Total bacterial count at 3 years was greater than a reference batch that was recently produced. Another study was conducted in the dark with the additive to detect protein degradation, as hydrolysis of thaumatin would result in a release of amino acids and small molecular weight proteins. During the period tested (36 months) no change in its appearance was observed, and no degradation of the thaumatin was detected. The results were the same in accelerated conditions (dark at 40 ± 1°C for 6 months). The stability of thaumatin in soft drinks (lemonade, cola and whole orange squash) was tested (visual examination and tasting) under refrigerated and room temperature conditions (5 and 20°C). Another stability study on the evolution of thaumatin stored for 8 months in a soft drink at pH 3 and in a powdered beverage at room temperature in the dark or light or under elevated temperature conditions (40°C) in the dark was performed. The thaumatin content did not change during the 8 months of storage for the samples at ambient temperature. In jellied sweets stored at ambient temperature (dark and light) and at 40°C in the dark, it was observed that thaumatin was degraded after 1 month in the elevated temperature study at 40°C, whereas, at ambient temperature, thaumatin was not detected after 3 months of storage in dark or light. Therefore, gelatin might account for the instability of thaumatin'.*

The other interested business operator did not perform new stability studies to support the re-evaluation of E 957. A compilation of references and old experimental studies (previously considered in the SCF evaluation of E 957 in 1985), supporting the stability of thaumatin (E 957) as dry powder, in aqueous polyol and acidic solutions, and when added in food products and beverages, were provided by the interested business operator (Documentation provided to EFSA nr: 4). According to such data and information, the interested business operator stated that dried thaumatin (E 957) is stable over 3

years of storage at 20°C with no notable changes in appearance, function, quality or analytical values (water content, ash, total bacterial count) except for a small increase in water content. Thaumatin (E 957) is also stable in a polyol solution 5% for a period of more than 5 years. Thaumatin (E 957) is particularly stable because of the tertiary structure of the protein with eight disulfide bonds that provide stability to heat and pH. Stability can be also influenced by other factors like oxygen, acidic polysaccharides and synthetic colours with acidic groups that can interact ionically with the basic thaumatin (E 957). Generally, the heat stability of thaumatin (E 957) increases at lower pHs, which allow thermal treatment at 100°C for hours at pHs between 3.5 and 5.5 without sweetness loss.

The thermostability of thaumatin (E 957) in aqueous solutions has been extensively studied, as documented in the peer-reviewed scientific literature. According to Coiffard et al. (1997), thaumatin degradation follows first-order kinetics and it is pH-dependent showing an optimum of stability at pH 2. In line with these findings, the paper by Kaneko and Kitabatake (2001) in which thaumatin (E 957) was tested over a pH range showed that the hydrolysis of peptide bonds, and other reactions, take place more slowly under acidic conditions. Kaneko and Kitabatake (1999) and Kaneko and Kitabatake (2001) found that thaumatin (E 957) aggregates upon heating at pH 7 above 70°C and this thermal aggregation is dependent on the concentration of thaumatin (E 957) and it is mainly induced by the formation of intermolecular disulfide bonds. In particular, thiol-catalysed disulfide interchange reactions contribute to the aggregation process and the disulfide interchange reaction is catalysed by cysteine, a free sulfhydryl residue formed via β-elimination of a disulfide bond. The thermostability of thaumatin (E 957) can be enhanced by the presence of certain polysaccharides. Couteau and Coiffard (2003) found an increase up to 20% in thaumatin stability following addition of gum arabic in E 957 formulations. Tang et al. (2021) have increased the resistance of thaumatin to thermal denaturation through protein engineering; saturation mutagenesis revealed that changing negatively charged amino acid residues to other non-negatively charged amino acids improved the thermal stability.

Thaumatin (E 957) has an isoelectric point around 12 (Van der Wel and Loeve, 1972) and at pH < 12, the protein is positively charged and can interact ionically with acidic polysaccharides, such as carrageenans that are negatively charged, resulting in a loss of sweetness (Ohashi et al., 1990, 1991).

3.2. Authorised uses and use levels

Maximum levels of thaumatin (E 957) have been defined in Annex II to Regulation (EC) No 1333/2008 on food additives, as amended. In this document, these levels are named maximum permitted levels (MPLs).

Currently, thaumatin (E 957) is an authorised food additive in the EU in 15 categories with MPLs ranging from 0.5 to 400 mg/kg and at *quantum satis* (QS) in the three food categories (FCs) of table-top sweeteners. Table 2 summarises the food categories with their restrictions/exceptions that are permitted to contain added thaumatin (E 957) and the corresponding MPLs as set by Annex II to Regulation (EC) No 1333/2008.

Table 2: MPLs of thaumatin (E 957) in foods according to the Annex II to Regulation (EC) No 1333/2008

Food category number	Food category name	E-number/ group	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
01.4	Flavoured fermented milk products including heat-treated products	E 957	Only as flavour enhancer	5
03	Edible ices	E 957	Only energy reduced or with no added sugar	50
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	E 957	Only energy reduced or with no added sugar	50
05.2	Other confectionery including breath refreshening microsweets	E 957	Only cocoa or dried fruit based, energy reduced or with no added sugar	50
		E 957	Only confectionery with no added sugar	50
05.3	Chewing gum[(a)]	E 957	Only with added sugar or polyols, as flavour enhancer	10

Food category number	Food category name	E-number/ group	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
		E 957	Only with no added sugar	50
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	E 957	Only confectionery with no added sugar	50
		E 957	Only cocoa or dried fruit based, energy reduced or with no added sugar	50
11.4.1	Table Top Sweeteners in liquid form	E 957		*Quantum satis*
11.4.2	Table Top Sweeteners in powder form	E 957		*Quantum satis*
11.4.3	Table Top Sweeteners in tablets	E 957		*Quantum satis*
12.6	Sauces	E 957	Only as flavour enhancer	5
14.1.4	Flavoured drinks	E 957	Only water-based flavoured non-alcoholic drinks, as flavour enhancer only	0.5
15.1	Potato-, cereal-, flour- or starch-based snacks	E 957	Only as flavour enhancer	5
16	Desserts excluding products covered in category 1, 3 and 4	E 957	Only as flavour enhancer	5
17.1	Food supplements supplied in a solid form, excluding food supplements for infants and young children	E 957	Only food supplements in chewable form	400
17.2	Food supplements supplied in a liquid form, excluding food supplements for infants and young children	E 957	Only food supplements in syrup form	400

MPL: maximum permitted level.
(a): If E 950, E 951, E 955, E 957, E 959 and E 961 are used in combination in chewing gum, the maximum level for each is reduced proportionally.

Thaumatin (E 957) is not authorised according to Annex III of Regulation (EC) No 1333/2008.

3.3. Exposure data

3.3.1. Concentration data

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued a public call[18] for concentration data (use levels and/or analytical data) on thaumatin (E 957).

In response to this public call, information on actual use levels of thaumatin (E 957) in foods was made available to EFSA by an interested business operator. No analytical data on the concentration of thaumatin (E 957) in foods were made available by the Member States.

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, actual use levels are required for performing a more realistic exposure assessment.

Reported use levels of thaumatin (E 957)

An interested business operator provided EFSA with one use level (100 mg/kg) of thaumatin (E 957) in foods belonging to FC 11.4.3 Table-Top Sweeteners in tablets, out of the 15 authorised uses according to Annex II to Regulation (EC) No 1333/2008 (Table 2).

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

The Mintel's GNPD is an online database which monitors new introductions of packaged goods in the market worldwide. It contains information of over 3 million food and beverage products of which

[18] https://www.efsa.europa.eu/sites/default/files/consultation/callsfordata/180122.pdf

more than 1,200,000 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.[19]

For the purpose of this Scientific Opinion, Mintel's GNPD[20] was used for checking the labelling of food and beverage products and food supplements for thaumatin (E 957) within the EU's food market as the database contains the compulsory ingredient information on the label.

According to Mintel's GNPD, thaumatin (E 957) was labelled on a few products (n = 34), mainly belonging to carbonated soft drinks, energy drinks, meal replacement drinks, food supplements, confectionery, table-top sweeteners and chewing gum, between January 2016 and May 2021.

Annex Table A3 lists the percentages of the food products labelled with thaumatin (E 957) out of the total number of food products per food subcategory according to Mintel's GNPD food classification. The percentages ranged from less than 0.1% in some food subcategories to 3.2% in Mintel's GNPD food subcategory 'Artificial sweeteners'. The average percentage of foods labelled to contain thaumatin (E 957) was 0.1%. However, these percentages may not consider the market share of the products listed per food category. Table A3 contains also the list of corresponding food categories; however, one-to-one linkage between Mintel subcategories and the food categories is not possible; thus, the list should be considered as an indicative approximation.

It was found that among the relevant subcategories of Mintel's GNPD, 10 products belonged to the food category 14.1.4 'Flavoured drinks', including a widely known brand of a zero calorie energy drink, a beverage concentrate and meal replacement drinks reported in year 2020. Other types of carbonated soft drinks were reported in 2016.

3.3.3. Food consumption data used for exposure assessment

EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (EFSA, 2011). The version of the Comprehensive database taken into account in this assessment was published in February 2020.[21] Data from EU member states were considered for the estimations.

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons may not be appropriate. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced owing to possible subjects' underreporting and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database includes the currently best available food consumption data across Europe.

Food consumption data from infants, toddlers, children, adolescents, adults and the elderly were used in the exposure assessment. For the present assessment, food consumption data were available from 40 different dietary surveys carried out in 23 European countries (Table 3). Not all countries provided consumption information for all age groups, and in some cases, the same country provided more than one consumption survey. For details on each survey, see Annex A, Table A.1.

Table 3: Population groups considered for the exposure estimates of thaumatin (E 957)

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From more than 12 weeks up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia
Toddlers[(a)]	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Slovenia, Spain
Children[(b)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Portugal, Spain, Sweden

[19] Missing Cyprus, Luxembourg and Malta.
[20] http://www.gnpd.com/sinatra/home accessed on 26/5/2021.
[21] Available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

Population	Age range	Countries with food consumption surveys covering more than 1 day
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Portugal, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
The elderly[(b)]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden

(a): The term 'toddlers' in the Comprehensive Database (EFSA, 2011) corresponds to 'young children' in Regulations (EC) No 1333/2008 and (EU) No 609/2013.
(b): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in Comprehensive Database (EFSA, 2011).

Since 2018, all consumption records in the Comprehensive Database are codified according to the FoodEx2 classification system (EFSA, 2015b). Nomenclature from the FoodEx2 classification system has been linked to the food categorisation system of Annex II of Regulation (EC) No 1333/2008, part D, to perform exposure assessments. In practice, the FoodEx2 food codes were matched to the food categories. For a detailed description of the methodology used to link FoodEx2 codes and the food categories, see section 5.2.1 of EFSA, 2020b. In FoodEx2, facets are used to provide further information about different properties and aspects of foods recorded in the Comprehensive Database than available in the basic food term, which defines the food itself. Facets have been used in the exposure assessment to further identify foods to be included in the assessment (i.e. foods in the relevant food categories with sweetener-related facets, see details in Annex A, Table A.2).

Food categories considered for the exposure assessment of thaumatin (E 957).

The food categories for which MPLs have been set including the FC 11.4 Table-top sweeteners for which a use level of thaumatin (E 957) was provided were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx2 classification system), at the most detailed level possible (up to FoodEx2 Level 7) (EFSA, 2015b).

Facets were used to identify eating events referring to foods assumed to contain sweeteners and to foods related to specific restrictions defined in the legislation (see details in Annex A, Table A.2).

Facets were not used to identify relevant eating events for FCs 11.4 Table-top sweeteners and 05.3 Chewing gum, and for gum drops in FC 05.2 Other confectionery including breath refreshening microsweets, energy drinks in FC 14.1.4 Flavoured drinks and vitamin and mineral supplements in FC 17 Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children. These foods and categories are expected to be major contributors to the exposure according to the literature and present a relatively high percentage of products labelled to contain at least one sweetener. Thus, all eating events referring to these foods and categories were included in the assessment as defined in the protocol (EFSA, 2020b).

As FC 17 does not consider food supplements for infants and toddlers as defined in the legislation, the exposure to thaumatin (E 957) from food supplements was not estimated for these two population groups.

Some restrictions/exceptions of certain food categories are not referenced in the EFSA Comprehensive Database, and therefore, the whole food category with the sweetener-related facets was considered in the exposure assessment. This was the case for five food categories (Annex A, Table A.2) for which the restriction 'for flavour enhancer only' could not be considered. This may have resulted in an overestimation of the exposure via the FCs 01.4 'Flavoured fermented milk products including heat-treated products', 12.6 'Sauces', 14.1.4 'Flavoured drinks', 15.1 'Potato-, cereal-, flour- or starch-based snacks' and 16 'Desserts excluding products covered in category 1, 3 and 4'.

Overall, out of the 15 FCs in which thaumatin (E 957) is authorised, 12 were included in the *regulatory maximum level exposure scenario* with their MPLs, while for the refined scenario, only the three FCs of 11.4 'Table-top sweeteners' were included with the reported use level for FCs 11.4.3 Table-top sweeteners in tablets.

3.4. Exposure estimate

3.4.1. Exposure to thaumatin (E 957) from its use as a food additive

The FAF Panel considered appropriate in the remit of the re-evaluation of sweeteners to estimate a chronic exposure. As suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011), dietary surveys with only 1 day per subject were not considered as they are not adequate to assess repeated exposure. Similarly, subjects who participated only 1 day in the dietary studies, when the protocol prescribed more reporting days per individual, were also excluded for the chronic exposure assessment.

Exposure assessments of sweeteners under the re-evaluation programme are carried out by the FAF Panel based on two different sets of concentration data: (a) MPLs of use set down in the EU legislation (in the regulatory maximum level exposure assessment scenario) and (b) use levels provided through calls for data (in the refined brand-loyal exposure assessment scenario).

To calculate chronic dietary exposure to thaumatin (E 957), food consumption and body weight data at the individual level were extracted from the Comprehensive Database and linked to the concentration data as described in section 5.2.1 of the Protocol (EFSA, 2020b).

Chronic dietary exposures were calculated by combining MPLs/use level of thaumatin (E 957) in each food with the average daily consumption for each food at individual level in each dietary survey and age class. Exposure estimates are divided by the individual's body weight resulting in a distribution of daily individual average exposures per kilogram body weight. On the basis of these distributions, the mean and 95th percentile (P95) exposures are calculated per survey and per age class. Mean estimates based on dietary surveys/age classes with less than six consumers and P95 estimated with less than 60 observations are not presented.

In this evaluation, as stated in the protocol (EFSA, 2020b), dietary exposures were assessed for consumers only of at least one food category containing thaumatin (E 957).[22] These population groups are expected to proxy exposure levels for the diabetics which are considered to be the population with the highest exposure to sweeteners. Moreover, in order to evaluate a possible underestimation of exposure, exposure estimates for consumers-only of an individual food category[23] was also calculated for the *refined brand-loyal exposure assessment scenario*. As reported use levels were only available for FC 11.4 Table top sweeteners, this gave the same result as for consumers only of at least one food category. Thus, exposure for consumers-only of an individual food category was also calculated for the *regulatory maximum level exposure assessment scenario*. These estimates are only reported in the exposure results section when they are higher than those for consumers only of at least one food category.

Regulatory maximum level exposure assessment scenario

The regulatory maximum level exposure assessment scenario was based on the MPLs as set in Annex II to Regulation (EC) No 1333/2008. For thaumatin (E 957), the MPLs used in the assessment are listed in Table 2. For the three food categories of 11.4 'Table-top sweetener' authorised according to QS, the maximum of the reported use level was used.

When specific food categories restrictions/exceptions defined in the legislation could not be identified in the consumption database, including the use as flavour enhancer, the highest MPL value among the subcategories was used for all.

MPLs/use level used in the above scenario is detailed in Table A.2 of Annex A.

Results of the regulatory maximum level exposure assessment scenario are not comparable to the refined brand-loyal scenario as the underlying population of consumers is different.

Refined brand-loyal exposure assessment scenario

The refined brand-loyal exposure assessment scenario to thaumatin (E 957) was based on use levels reported by the interested business operator. This exposure scenario considers only those food categories for which these data were provided to the Panel. In the brand-loyal consumers only

[22] Part of the survey population may have no exposure to the additive because they did not report eating a food from one of the food categories that could contain the additive (these are 'non-consumers'). The subgroup who report eating these foods are called consumers.

[23] Similarly, if the focus is not only on the whole diet but on estimates of exposure for each FC separately, again there will be part of the survey population that did not report eating that FC and the other part of the population who did report eating that single FC, again being referred to as the 'consumers-only'.

scenario, it is assumed that a consumer is exposed long-term to thaumatin (E 957) present at the maximum reported use for one food category.

This exposure estimate was calculated with the use level (100 mg/kg) provided for the only FC 11.4.3. Table top sweeteners in tablets, extrapolated to FC 11.4 Table top sweeteners.

Dietary exposure for consumers of one or more food categories containing thaumatin (E 957)

Table 4 summarises the estimated exposure to thaumatin (E 957) from its use as food additive in six population groups (Table 3) according to the different exposure scenarios among consumers only of at least one food containing thaumatin (E 957). Detailed results per population group and survey are presented in Annex A Table A.4.

Table 4: Summary of dietary exposure to thaumatin (E 957) from its use as food additive in the regulatory maximum level exposure assessment scenario and in the refined brand-loyal exposure assessment scenario, in six population groups among consumers only of at least one food containing thaumatin (E 957) (minimum–maximum across the dietary surveys in mg/kg bw per day)

	Infants (12 weeks–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Regulatory maximum level exposure assessment scenario						
• Mean[(a)]	< 0.01–0.01	0.01–0.04	< 0.01–0.07	< 0.01–0.08	< 0.01–0.05	< 0.01–0.03
• 95th percentile[(b)]	0.03	0.01–0.09	0.01–0.29	0.01–0.33	< 0.01–0.27	< 0.01–0.11
Refined brand-loyal exposure assessment scenario						
• Mean[(a)]	No exposure	No exposure	< 0.01	< 0.01–0.01	< 0.01	< 0.01
• 95th percentile[(b)]	No exposure	No exposure	< 0.01	< 0.01	< 0.01–0.02	< 0.01–0.01

(a): Mean estimates obtained on dietary surveys/age classes with less than 6 consumers may not represent the population group, thus not presented in this table.
(b): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (ref to protocol). Those estimates were not included in this table.

For the regulatory maximum level exposure assessment scenario, the highest mean exposure was found in adolescents (0.08 mg/kg bw per day) as well as the highest P95 (0.33 mg/kg bw per day).

In the refined brand-loyal exposure assessment scenario, in case of infants, no consumption of table-top sweeteners was reported in the Comprehensive database; thus, they have no exposure estimated. Similarly, for toddlers, no exposure is presented as no surveys included 6 or more consumers in this population group. Highest mean exposure was found in adolescents (0.01 mg/kg bw per day) while highest P95 was 0.02 mg/kg bw per day in adult consumers.

Main food categories contributing to the exposure to thaumatin (E 957)

In case of the refined brand-loyal exposure assessment scenario, the exposure was only coming from the consumption of table-top sweeteners.

The main contributor in the regulatory maximum level exposure assessment scenario from Table 4 (Annex A, Table A.5) was FC 17 Food supplements in all age groups (except infants and toddlers where it is not authorised), while FC 14.1.4 in several surveys in almost all age classes. For elderly, FC 11.4 Table-top sweeteners also contributed significantly in adults and the elderly, while FC 5.2 Other confectionery contributed significantly in adults, children, and toddlers.

In infants, there were very few consumers of foods containing thaumatin (E 957) in general in all relevant categories, except in Denmark (n = 79) where the main contributor was FC 5.2 Other confectionery.

In toddlers, the three main contributing categories were FCs 1.4 Flavoured fermented milk products, 14.1.4 Flavoured drinks and 5.2 Other confectionery.

Dietary exposure for consumers of individual food categories containing thaumatin (E 957)

In the case of thaumatin (E 957) where only one use level was provided for table top sweeteners, refined brand-loyal exposure assessment scenario estimated for consumers of table-top sweeteners is based on the same population and occurrence data than the refined brand-loyal exposure assessment scenario for consumers of one or more food categories containing thaumatin (E 957), the estimates are the same and are not repeated here.

Among consumers only populations of food categories separately (Annex A, Table A.6), in the regulatory maximum level exposure assessment scenario, the following exposure results exceeded the highest results of consumers of one or more food categories (Table 4):

- mean exposure results in consumers only of FC 05.1 Chocolate and chocolate products in children (0.17 mg/kg bw per day) and adolescents (0.10 mg/kg bw per day); and of FC 17 Food supplements in adolescents (0.10 mg/kg bw per day) and elderly (0.09 mg/kg bw per day).
- P95 exposure results in consumers only of FC 05.1 Chocolate and chocolate products in children (0.48 mg/kg bw per day) and of FC 17 Food supplements in adolescents (0.34 mg/kg bw per day).

3.4.2. 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 uncertainties have been considered and summarised in Table 5.

Table 5: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction[(a)]
Consumption data	
Different methodologies/representativeness/underreporting/misreporting/no portion size standard/only a few days	+/–
Underreporting of food descriptors (facets) concerning the presence or potential presence of sweeteners	n.a.
Regulatory maximum level exposure assessment scenario: Lack of the possibility to apply some of the restrictions specified in the legislation (flavour enhancer only) In the cases when flavour enhancer was applied in products not labelled as reduced energy	+[(b)] –
Use of the additive in table-top sweeteners added to home-made products might not be captured	–
Concentration data	
Refined brand-loyal exposure assessment scenario: The food additive is assumed not to be used in the food categories in which it is authorised and for which no use levels were submitted	–
Refined brand-loyal scenario: a number of Mintel subcategories in which thaumatin (E 957) was labelled were included in the current exposure assessment: 3 out of 15 subcategories, representing 4% of the products labelled with thaumatin (E 957)	–
Use levels/MPLs considered applicable to all foods within the entire food category, while the percentage of the foods in a subcategory labelled with thaumatin (E 957) in Mintel was maximally 3.2%	+
Regulatory maximum level exposure assessment scenario: Exposure calculations are based on the MPLs/maximum reported use levels	+
Regulatory maximum level exposure assessment scenario: 15 food categories out of the 9 that are represented in Mintel were considered in the assessment	+
Methodology	
Regulatory maximum level exposure assessment scenario cannot be fully compared to the refined brand-loyal scenario as the two scenarios are performed over different populations of consumers only	+/–
Use of data from food consumption survey covering only a few days to estimate high percentile (95th) of long-term (chronic) exposure	+

n.a.: not applicable.
(a): +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.
(b): Uncertainty considerations on the direction (+/–) are made assuming the effect on the same underlying population of consumers.

Overall, the Panel considered the exposure to thaumatin (E 957) from its use as food additive, in European countries present in the Comprehensive Database to be an overestimation for the regulatory maximum level exposure scenario, and an underestimation for the refined scenario.

An underestimation in the refined scenario was due to the overall uncertainties (Table 5), in particular because use level data were only available for table-top sweeteners, while according to Mintel's GNPD, thaumatin (E 957) is used in flavoured drinks (especially in energy drinks), food supplements and a small number of other subcategories as well (Annex A, Table A.3).

3.5. Biological and toxicological data

The biological and toxicological studies that were assessed as relevant to this opinion, according to the inclusion criteria established in the draft protocol on hazard identification and characterisation of sweeteners (EFSA, 2020a), are listed in Appendix B. The identified studies were provided to EFSA following the public call for biological and toxicological data.[2] Additional relevant data were also identified from the literature. A list of studies that did not meet the inclusion criteria is provided in Annex B.

Studies considered to be relevant for the safety assessment of thaumatin (E 957), including short-term, sub-chronic and prenatal developmental toxicity in animals as well as human studies, are summarised in Appendix C. For these studies, an evaluation of the risk of bias (RoB) was performed, according to the criteria and rules outlined in the draft protocol (EFSA, 2020a, Tables 8 and 9). The risk of bias evaluation is reported in Appendix A (Tables A.1 and A.2). In addition, a weight of evidence (WoE) approach was applied for each health outcome in the collection of animal and human studies (NTP-OHAT, 2019).

For studies on acute toxicity and genotoxicity, the review approach was narrative. Information available as regards thaumatin digestibility and allergenicity (with route of exposure different from oral) were also summarised as a narrative (3.5.5 Other studies).

No reproductive and chronic toxicity or carcinogenicity studies were submitted by the interested parties and none were retrieved from the literature.

3.5.1. Absorption, distribution, metabolism and excretion

Only data on thaumatin digestibility were received from the interested business operators and no new data were identified in the literature. No other data on absorption, distribution, metabolism and excretion (ADME) were available.

3.5.1.1. Stability of the compound in the GI tract

In vitro protein digestibility

The original study reports from two *in vitro* digestibility studies were submitted (Documentation provided to EFSA nr: 3 and 7). In the first study (Higginbotham 1978, in Documentation provided to EFSA nr: 7), the *in vitro* digestibility of thaumatin was compared to that of egg albumin. In the second more recent study (Wageningen Food & Biobased Research, 2020 in Documentation provided to EFSA nr: 3), the *in vitro* digestibility of thaumatin was compared to that of whey protein. Thaumatin had a similar digestibility score to whey protein and egg albumin. SDS-PAGE analysis was also performed and confirmed that thaumatin was readily hydrolysed after digestion with pepsin and pancreatin.

Overall, the Panel considered that the studies showed that thaumatin was digested to at least a similar extent as egg albumin or whey protein *in vitro*.

In vivo protein digestibility

The original study report from an *in vivo* digestibility study in rats was submitted (Edwards, 1981 in Documentation provided to EFSA nr: 7). In the study, 20 male Sprague-Dawley rats, 140–160 g bw, were housed singly with feed and water available *ad libitum*. Rats were randomly selected (10/group) and fed a diet containing 10% protein solely from either egg albumin followed by a mixture of 5% egg albumin and 5% thaumatin (both for 10 consecutive days) or fed identically but in the reverse order.

Each 10-day period consisted of a 5-day period for the animals to acclimatise, followed by a 5-day period during which urine and faeces were collected and analysed for nitrogen content. Body weights and food intakes were monitored. Irrespective of the order in which the diets were provided within each 10-day period, rats receiving thaumatin and albumin consumed less feed and gained less weight than those receiving albumin alone. Dietary protein digestion was estimated by the authors to be at approximately 90% (mean albumin and thaumatin digestibility 89.4% and 90.6%, respectively).

The Panel considered that thaumatin is readily digestible in rats. The Panel also considered that the lower growth rates in rats fed thaumatin in this study were due to the reduced feed intake and possibly also due to some nutritional imbalance, rather than an adverse effect of thaumatin.

3.5.2. Acute toxicity

The original reports of two acute toxicity studies were submitted through the call for data (Ben-Dyke 1975; Ben-Dyke and Joseph, 1976 in Documentation provided to EFSA nr: 7). No signs of toxicity or mortality were observed up to 20 and 21 g/kg bw in rats and mice, respectively.

3.5.3. Genotoxicity

Two original study reports on genotoxicity of thaumatin were received through the call for data (Higginbotham, 1980; Tesh et al., 1977a in Documentation provided to EFSA nr: 7). Detailed description of the studies is reported in Appendix D.

Thaumatin was tested with negative results in a limited bacterial mutation assay (Higginbotham, 1980) and in the dominant lethal test in mice (Tesh et al., 1977a). Both tests were performed before the development of OECD guidelines for GLP and genotoxicity testing. These studies were previously evaluated by the SCF (1985) and JECFA (1986) and neither Committee raised a concern with respect to genotoxicity. In this respect, the Panel noted that the available data set (Ames test and dominant lethal assay) is not aligned with current requirements for genotoxic hazard identification (Ames test and *in vitro* micronucleus assay, according to the EFSA Scientific Committee, 2011), as the Ames test was performed with a too limited protocol, and the dominant lethal assay is appropriate only for the follow-up at the germ cell level of substances that are clastogenic/aneugenic in somatic cells *in vivo*. However, the Panel also noted that the active principles of thaumatin consist of two high molecular weight digestible proteins (thaumatin I and II), for which no genotoxic properties can reasonably be anticipated (Thybaud et al., 2016; WHO, 2020). In addition, the Panel noted that the non-proteinaceous fraction present in E 957 consists of carbohydrates, residual from the plant material which hydrolyse to simple monosaccharides with no structural alerts for DNA binding and *in vitro* genotoxicity (OECD QSAR ToolBox, version 4.4.1), and inorganic salts (e.g. sulfated ashes), which also do not raise concern for genotoxicity (EFSA FAF Panel, 2019).

Overall, the Panel concluded that there is no concern with respect to genotoxicity for thaumatin (E 957).

3.5.4. Synthesis of systematically appraised evidence on biological and toxicological effects

Annex C reports the evaluated animal studies, rated as Tier 1 and Tier 2 in the risk of bias evaluation, that were then gathered by endpoint within the different health outcome categories for which a WoE analysis was performed. As regards human data, only Tier 3 studies were available and were considered in the WoE analysis (Annex D). A narrative synthesis is reported below.

3.5.4.1. Animal studies

The following animal oral toxicity studies have been considered (Table 6): a 4-week toxicity study in rats (Danks et al., 1984), focusing on thyroid function, performed with a single dose of 0 or 3% of thaumatin in the diet, equivalent to 0 or 3600 mg/kg bw per day; a 13-week toxicity study in rats (Ben-Dyke et al., 1976) performed with 0, 1, 4 and 8% of thaumatin in the diet (equal to 0, 696, 2,608, 5,200 and 0, 889, 3,350, 6,608 mg/kg bw per day in males and females, respectively); a 13-week toxicity study (Hiscox et al., 1978) in rats performed with 0, 0.3, 1 and 3% thaumatin in the diet (equal to 0, 256, 748, 2,418 and 0, 293, 998, 2,822 mg/kg bw per day in males and females, respectively); a 13-week toxicity study in rats (Hagiwara et al., 2005) performed with 0, 0.3, 1 and 3% of irradiated thaumatin in the diet (equal to 0, 260, 788, 2,502 and 0, 299, 1,042, 2,889 in males and females, respectively) or with 0 or 3% only for non-irradiated thaumatin in the diet (equal to 0 or

2,394 and 0 or 2,925 mg/kg bw per day in males and females, respectively); a 13-week toxicity study in dogs (Barker et al., 1981) performed with 0, 0.3, 1 and 3% thaumatin in the diet (equal to 0, 133, 435, 1,305 and 0, 139, 469, 1,476 mg/kg bw per day in males and female, respectively); and a prenatal developmental toxicity study (gavage) in rats (Tesh et al., 1977b) performed with 0, 200, 600, 2,000 mg/kg bw per day.

Table 6: Summary table of animal oral toxicity studies

RefID	Authors	Study type	Exposure duration	Species	Dose level (% in the diet)	Dose level (mg/kg bw per day)
1429	Danks et al. (1984) (Documentation provided to EFSA nr. 7)	Short-term toxicity	4 weeks	Rat	0; 3	0; 3,600 (M, F)
1430	Ben-Dyke et al. (1976) (Documentation provided to EFSA nr. 7)	Sub-chronic toxicity	13 weeks	Rat	0; 1; 4; 8	0; 696; 2,608; 5,200 (M) and 0; 889; 3,350; 6,608 (F)
1425	Hiscox et al. (1978) (Documentation provided to EFSA nr. 7)	Sub-chronic toxicity	13 weeks	Rat	0; 0,3; 1; 3	0; 256; 748; 2,418 (M) and 0; 293; 998; 2,822 (F)
1435	Hagiwara et al. (2005)	Sub-chronic toxicity	13 weeks	Rat	0; 0,3; 1; 3	0; 260; 788; 2,502 or 2,394 [(a)] (M) and 0; 299; 1,042; 2,889 or 2,925 [(a)] (F)
1433	Barker et al. (1981) (Documentation provided to EFSA nr. 7)	Sub-chronic toxicity	13 weeks	Dog	0; 0,3; 1; 3	0; 133; 435; 1,305 (M) and 0; 139; 469; 1,476 (F)
1420	Tesh et al. (1977b) (Documentation provided to EFSA nr. 7)	Prenatal developmental toxicity	GDs 6–15	Rat	- (gavage)	0; 200; 600; 2,000 (F)

M: males; F: females; GDs: gestational days.
(a): For non-irradiated thaumatin, animals were exposed to one dose only of 3% of test substance in the diet, equal to 2,394 and 2,925 mg/kg bw per day in males and females, respectively.

The health outcome categories reported from the animal studies, each encompassing of a number of endpoints, are illustrated in Table 7.

Table 7: Health outcomes and related endpoints of the systematically appraised animal studies subjected to WoE analysis

Health outcomes	Endpoints
General toxicity	Clinical signs; body weight; feed intake; water intake; clinical chemistry; ophthalmoscopic examination
Haematotoxicity	Haemoglobin concentration; erythrocyte count; haematocrit
Liver toxicity	Liver weight; macro- and histopathology of liver; liver function (bromosulfthalein dye retention test); clinical chemistry
Nephrotoxicity	Kidney weight; macro- and histopathology of kidney; kidney function (6-h urine concentration test); urinalysis; clinical chemistry
Other organs toxicity[(a)]	Macro- and histopathology of mammary gland, lung and other organs[(b)]
Endocrine toxicity	Plasma triiodothyronine (T3) and thyroxine (T4); thyroid weight, macro- and histopathology of thyroid; pituitary weight; adrenal weight

Health outcomes	Endpoints
Developmental toxicity	Pre-implantation loss, post-implantation loss, litter size, fetal weight, fetal external, skeletal and visceral abnormalities
Immunotoxicity and allergenicity (oral route)	Serum antibodies; spleen weight; macro- and histopathology of spleen

(a): The organs included for assessment will vary depending on study-type and sweetener in question.
(b): Organs that are examined in standard sub-chronic toxicity studies other than adrenals, kidney, liver, pituitary, spleen, thyroid already considered under other health outcomes (typically for sub-chronic studies: e.g. aorta, bone marrow, brain, caecum, cervical and mesenteric lymph node, duodenum, epididymis, eye and optic nerve, heart, ileum, oesophagus, ovaries, pancreas, prostate, salivary glands, seminal vesicles, sciatic nerve, skeletal muscle, skin, spinal cord, stomach, testes, thymus, tongue, trachea, uterus, urinary bladder).

The Panel based its evaluation on the final ratings and comments presented in the WoE tables for each health outcome category (Annex C).

General toxicity

The following endpoints were assessed in the short-term, sub-chronic, prenatal developmental toxicity studies: clinical signs, bodyweight, feed and water intake, clinical chemistry, ophthalmological changes. There were no changes in clinical signs and no adverse ophthalmological findings. The changes observed in feed intake and bodyweight in the sub-chronic toxicity studies in rat (Ben-Dyke et al., 1976, Hiscox et al., 1978, Hagiwara et al., 2005) and dog (Barker et al., 1981) were within the normal range, were not consistent across study type or sex and were not dose dependent. Where reduced feed intake and body weight gain were observed, they were considered a consequence of reduced feed palatability. Water intake was not affected. Clinical chemistry values varied within the normal range, and there were no dose-related effects in any species. The highest doses tested (Table 6) were considered to be NOAELs in all studies.

Haematotoxicity

Reported changes in haemoglobin concentration, erythrocyte count and haematocrit in three sub-chronic studies in rat and dog (Ben-Dyke et al., 1976, Hiscox et al., 1978, Barker et al., 1981) were within the normal ranges for these species. In one sub-chronic study in rat (Hagiwara et al., 2005), no treatment-related adverse effects were reported (data not presented in detail by the authors). The highest doses tested (Table 6) were considered to be NOAELs in all studies.

Liver toxicity

Liver toxicity was assessed by the following endpoints: liver weight, macro- and histopathology, function and related clinical chemistry parameters. Data were reported in four sub-chronic toxicity studies in rat and dog. Increases in absolute and/or relative liver weights were observed in rats (up to 17% but inconsistent between sexes) (Ben-Dyke et al., 1976, Hiscox et al., 1978, Hagiwara et al., 2005) and dogs (up to 22%, males only) (Barker et al., 1981) following intake of the highest dose tested (5,200 and 6,608 mg/kg bw per day in rat and 1,305 and 1,476 mg/kg bw per day in dog, in males and females, respectively). The findings were not accompanied by treatment-related histopathological or clinical chemistry value changes indicative of liver toxicity. In the absence of treatment-related histopathological and clinical chemistry changes indicative of liver injury, the increases in absolute and/or relative liver weights were likely adaptive changes and not adverse. The highest doses tested (Table 6) were considered to be NOAELs in all studies.

Nephrotoxicity

The endpoints for kidney toxicity were kidney weight, macro- and histopathology, function, urinalysis and related clinical chemistry parameters. Four sub-chronic toxicity studies in rat and dog were assessed. In one study in rats (Hiscox et al., 1978), a dose-dependent increase (up to 8%) was observed in absolute and relative kidney weight in females only (293, 998, 2,822 mg/kg bw per day). However, these changes were within the normal range and were not accompanied by any toxicologically relevant histopathological findings. In male dogs only (Barker et al., 1981), an increase (up to 19%) was observed in absolute kidney weight, but not in the relative weight following intake of 133 mg/kg bw per day, the lowest dose tested. Urinalysis and relevant clinical chemistry parameters showed no effect across studies and species. Changes in absolute and relative kidney weight were

inconsistent and not supported by any changes in kidney function or histopathology, and it is concluded that there were no adverse effects on the kidney. The highest doses tested (Table 6) were considered to be NOAELs in all studies.

Other organ toxicity

No substance-related organ pathology, including in testis, ovary and uterus, was reported in the rat and dog sub-chronic or prenatal developmental toxicity studies (Ben-Dyke et al., 1976, Hiscox et al., 1978, Hagiwara et al., 2005, Barker et al., 1981). The highest doses tested (Table 6) were considered to be NOAELs in all studies.

Endocrine toxicity

The following endpoints from one short-term and four sub-chronic toxicity studies in rat and dog were assessed: plasma T4 (thyroxine) and T3 (triiodothyronine), thyroid weight, thyroid macro- and histopathology, pituitary and adrenal weights. Plasma T4 and T3 were measured only in the short-term study. No effects were observed on the hormone levels up to 3,600 mg/kg bw per day (one dose only) (Danks et al., 1984). In one sub-chronic toxicity study in rat (Hiscox et al., 1978), a dose-dependent increase (up to 69%) in absolute and relative thyroid weights was observed in males at all doses tested. In females, both absolute and relative thyroid weights were decreased by up to 50% at all doses compared to controls. The changes in thyroid weights were not accompanied by adverse histopathological findings. In another sub-chronic toxicity study in rat (Ben-Dyke et al., 1976), absolute and relative thyroid weight increases (up to 42.4%) were observed only in females at the mid dose of 3,350 mg/kg bw per day. No adverse histopathological findings were observed at any dose. In another sub-chronic toxicity study in rat (Hagiwara et al., 2005), results were incompletely presented. Furthermore, in the sub-chronic toxicity study in dog (Barker et al., 1981), no effects on thyroid weight and histopathology were observed at any dose. As regards the pituitary and adrenals weights, no toxicologically relevant changes were observed in rat or dog. The highest doses tested (Table 6) were considered to be NOAELs in all studies.

Developmental toxicity

A single prenatal developmental toxicity study (GDs 6–15) in rats (Tesh et al., 1977b) was assessed for the following endpoints: pre-and post-implantation loss, litter size, fetal weight and fetal external, skeletal and visceral abnormalities. No adverse effects were observed at any dose up to 2,000 mg/kg bw per day.

Immunotoxicity and allergenicity (oral route)

Serum antibodies, spleen weight and macro- and histopathology were assessed in three rat and one dog sub-chronic toxicity studies. Serum antibodies were measured in one rat study only (Ben-Dyke et al., 1976) without detection of antibodies to thaumatin. A reduction in absolute spleen weight (about 19%) was reported in female dogs at the low dose (Barker et al., 1981). In a sub-chronic toxicity study in rats (Ben-Dyke et al., 1976), absolute spleen weight at the low dose and relative spleen weight at low and mid dose were observed in females only. These effects were not dose dependent; no histopathological correlation of the findings was observed, and therefore, they were not considered adverse (Sellers et al., 2007). The highest doses tested (Table 6) were considered to be NOAELs in all studies.

Overall rating of confidence in the body of evidence for the endpoints and conclusions of evidence for health effects

Overall, the Panel considered the confidence in evidence to be moderate for the absence of adverse effects of thaumatin (E 957) in doses up to 5,200 mg/kg bw per day, tested in male rats. The risk of bias evaluation raised doubts about some of the results. In addition, there was uncertainty about the precision of some of the outcomes due to lack of statistical information and the use of small group sizes (Table 8). This rating results in a moderate evidence of no health effect (Table 9 and see also Appendix A).

Table 8: Summary table of health outcomes and WoE ratings

	Initial rating	Downgrading evaluation				Upgrading evaluation			Final rating of confidence
		Risk of Bias	Unexplained inconsistency	Relevance of endpoints	Imprecision	Magnitude of effect	Dose-response	Consistency across species/study design	
General toxicity	High	Downgrade	Not serious	Not serious	Not serious	Not large	No	Upgrade	High
Haematotoxicity	High	Downgrade	Not serious	Not serious	Not serious	Not large	No	Upgrade	High
Liver toxicity	High	Not likely bias	Not serious	Not serious	Downgrade	Not large	No	Upgrade	High
Nephrotoxicity	High	Not likely bias	Not serious	Not serious	Downgrade	Not large	No	No	Moderate/ High
Other organs toxicity	High	Downgrade	Not serious	Not serious	Not serious	Not large	No	Upgrade	High
Endocrine toxicity	High	Downgrade	Not serious	Not serious	Not serious	Not large	No	Upgrade	High
Developmental toxicity	High	Downgrade	Not serious	Not serious	Downgrade	Not large	No	Upgrade	Moderate
Immunotoxicity and allergenicity via oral route	High	Not likely bias	Not serious	Not serious	Not serious	Not large	No	Upgrade	High

The following terms were used to express the levels of confidence in the body of evidence for an association between exposure to the substance and the health outcome(s). An initial confidence rating was assigned based on study design as 'High', 'Moderate', 'Low' or 'Very low'. The final rating of the confidence in the body of evidence was either 'High', 'Moderate', 'Low' or 'Very low/No evidence identified'.

For translating the confidence rating into a level of evidence for (no) health effect, see Figure A.2 in Appendix A. In the case of no health effect, the elements with highest uncertainty will be given more weight into the overall rating for all outcomes.

Table 9: Translation of confidence rating into level of evidence for (no) health effect

	Initial rating	Downgrading evaluation				Upgrading evaluation			Final rating of confidence	Presence of health effects?	Rating for no health effect
		Risk of Bias	Unexplained inconsistency	Relevance of endpoints	Imprecision	Magnitude of effect	Dose-response	Consistency across species/study design			
Overall rating for all outcomes	High	Downgrade	Not serious	Not serious	Downgrade	Not large	No	Upgrade	Moderate	No	**Moderate**

The following terms were used to express the levels of confidence in the body of evidence for an association between exposure to the substance and the health outcome(s). An initial confidence rating was assigned based on study design as 'High', 'Moderate', 'Low' or 'Very low'. The final rating of the confidence in the body of evidence levels was either 'High', 'Moderate', 'Low' or 'Very low or No evidence identified'. Correspondingly, the levels of evidence for the presence of a health effect could be denoted 'High'; 'Moderate'; 'Low' or 'Inadequate'. When no health effect was reported, the level of evidence was rated as either 'Evidence of no health effect', 'Moderate' or 'Inadequate' (for both 'Low' and 'Very low/No evidence identified') (more details in Figure A.2 of Appendix A).

3.5.4.2. Human studies

Three human intervention studies conducted in healthy volunteers (non-peer-reviewed reports) examining possible effects of oral exposure to thaumatin on blood chemistry and allergenicity were submitted through the call for data (Tompkins and Enticknap, 1984; MacLeod et al., 1981; Eaton et al., 1981 in Documentation provided to EFSA nr: 7). These studies were conducted in the early 1980s when conduct and reporting were considerably different from current standards. No signs of overt toxicity were observed in a study assigning 15 subjects to 280 mg thaumatin/day to thaumatin over 13 weeks and comparing them to same number of controls (Tompkins and Enticknap, 1984). No sign of allergenicity assessed by skin prick test after oral exposure to thaumatin was observed in two studies that combined recruited 35 subjects working in a thaumatin production facility (MacLeod et al., 1981, Eaton et al., 1981).

Overall rating of confidence in the body of evidence for the endpoints and conclusions of evidence for health effects

All three studies were associated with a high risk of bias (Tier 3) and underpowered. Due to the low confidence in the findings based on the weight of evidence analysis, no conclusion could be drawn on the effects in human from this limited data set (Annex D).

3.5.4.3. Hazard identification conclusion based on integration of human and animal evidence

In combination, the body of evidence from both human and animal studies presents a consistent pattern of findings indicating that oral intake of thaumatin is not associated with any health effects. The evidence for an absence of effect was moderate for some outcomes. Hence, there is moderate evidence that exposure to thaumatin is not associated with the investigated health outcomes.

3.5.5. Other studies

Allergenicity via route of exposure different from oral

Although the route of exposure is different from oral, the following studies were considered relevant to be included in this opinion.

Animal studies

The potential for thaumatin to induce an allergic response was evaluated *in vivo* in three different animal species (Stanworth et al., 1977 in Documentation provided to EFSA nr: 7).

Guinea pig

In the first study, five animals (sex not reported) were sensitised by an intramuscular injection of either thaumatin or egg albumin (50 mg each) together with 0.1 mL of either Freund's adjuvant or 1.3% alhydrogel (incomplete adjuvant). After treatment, guinea pigs were sacrificed at intervals between 12 and 30 days and three sections of ileum from each animal were challenged with a range of doses of either thaumatin or egg albumin. Contractions were recorded and expressed as a percentage of the maximum activation induced by a standard histamine solution. In animals injected with incomplete adjuvant, the response was similar for both proteins, while thaumatin was found to be more active when injected with Freund's complete adjuvant. The test on ileum preparations of sensitised guinea pigs revealed that the minimum dose of thaumatin able to elicit a response was 250 ng which was comparable to the minimum dose of egg albumin needed to evoke an anaphylactic response in the gut of control animals sensitised with egg albumin.

Rat

Five male rats were sensitised by a subcutaneous injection of thaumatin or egg albumin (10 μg each) with Freund's adjuvant at four separate sites. Rats were euthanised 11 or 12 days after treatment and the levels of anaphylactic antibody in the serum were determined by passive cutaneous anaphylactic (PCA) assay. It was observed that anaphylactic antibody titres in rats were lower for thaumatin than ovalbumin.

Baboon

After an intravenous injection of Evan's blue dye (indicator dye), one baboon received an intradermal injection of thaumatin at a concentration range of 10^{-2}–10^{-9} M. The blueing response (mm^2) was recorded, showing a weak response compared with the responses obtained with isotonic saline solution.

In summary, the results of these studies showed that thaumatin applied intramuscularly, subcutaneously and intravenously has allergenic potential similar to that of egg albumin.

Observational studies in humans

From the literature search, one observational study was conducted in a small cohort of occupationally exposed individuals reporting allergic symptoms in the upper airways. In a chewing gum production plant, four cases out of eight who were regularly exposed to a powder mixture of 10% of thaumatin and 90% gum arabic had allergic signs (nasal concha hypertrophy) and symptoms of upper respiratory allergy (e.g. rhinorrhoea, nasal obstruction, sneezing and nasal concha hypertrophy). All symptomatic workers had a positive response in a skin prick test for pure thaumatin and two of them were also positive for pure gum arabic and gum arabic-specific IgE. The presence of atopy (positiveness in the skin prick test for grass, alder, birch, house dust mites) was found in three workers diagnosed with rhinitis. The exposed asymptomatic workers (n = 4) presented distinct goblet and basal cell hyperplasia and two of them presented also squamous metaplasia. After this evidence, the chewing gum production plant substituted the powdered thaumatin with a liquid form and suspended exposure to gum arabic powder. Following this intervention, workers with rhinitis became free or almost free of symptoms. In two of the workers exhibiting only allergy to thaumatin in the nasal cavity concha hypertrophy also returned to normal while in exposed and asymptomatic workers marked regression of the goblet and basal cell hyperplasia and disappearance of squamous metaplasia was observed in the following 2 years. The results of the study suggest that exposure at occupational level to both thaumatin and gum arabic powder may cause rhinitis, in particular among susceptible individuals, and nasal mucosa signs of allergy (Tschannen et al., 2017).

Summarising the findings on human and animal studies conducted to establish the safety of thaumatin (Higginbotham et al., 1983), reported results from a cross-sectional study (n = 140) conducted in workers occupationally exposed (up to 7 years) to thaumatin. Although not explicitly reported, the main route of exposure was most likely via inhalation. These subjects were screened to common inhalant allergens by skin-prick testing. Out of 140 subjects tested, 13 subjects showed positive response to thaumatin.

Based on the findings reported in occupationally exposed workers, the Panel concluded that thaumatin may elicit an allergic response via inhalation.

3.6. Environmental considerations

An extensive review collating published data on artificial sweeteners including thaumatin (E 957) to identify evidence of potential adverse effect on the environment was performed (Agriculture and Environment Research Unit, University of Hertfordshire, 2021). Data relevant to address the potential effects on the environment of thaumatin (E 957) or its metabolites and degradation products were not available. Considering that thaumatin is a digestible protein a concern for the environment is not expected.

4. Discussion

Thaumatin (E 957) is authorised as a food additive in the EU in accordance with Annex II to Regulation (EC) No 1333/2008 on food additives and its specifications are defined in the Commission Regulation (EU) No 231/2012.

Thaumatin was previously assessed by both JECFA (JECFA, 1983, 1986) and the SCF (SCF, 1985, 1989). Following these evaluations, thaumatin (E 957) was considered acceptable for use and the ADI was established as 'not specified'. A more recent opinion on the safety of thaumatin for use as a feed additive concluded that there were no concerns for consumer safety from the use of thaumatin in feed and water for drinking for all species, as '*thaumatin is a highly digestible protein and no residues in edible tissues/products are expected*' (EFSA FEEDAP Panel, 2011). In 2015, the former EFSA ANS Panel issued a scientific opinion on a proposed extension of use of thaumatin (E 957) concluding that,

based on the existing toxicological evaluations, the proposed extension of uses and changes to use levels would not represent a safety concern.

As specified in Commission Regulation (EU) No 231/2012, thaumatin (E 957) is obtained by acidic aqueous extraction of the arils of the fruit of *Thaumatococcus daniellii* (Benth) and consists essentially of the proteins thaumatin I and thaumatin II, together with minor amounts of plant constituents, such as arabinogalactan and arabinoglucuronoxylan polysaccharides, derived from the source material.

According to the information provided by the interested business operators, thaumatin (E 957) is commercially extracted with a purity of not less than 94% and it can be further purified (by ultrafiltration) to above 97%. The Panel observed that the methodology currently indicated in the EU specification for the determination of the minimum purity assay for E 957, i.e. not less than 93% proteins, is based on the nitrogen content and then converted to percentage of the total proteins using NCF of 6.2 (Kjeldahl method). Upon a clarification request regarding the appropriateness of the use of an NCF, one interested business operator indicated that an NCF of 5.8 rather than of 6.2 would be appropriate if the proteins in E 957 were solely thaumatin I and thaumatin II. The results of SDS-PAGE analysis in one commercial batch of E 957 showed that the food additive is not only comprised of the thaumatin proteins but also of other proteins and/or peptides. In this particular case, thaumatin I and II proteins accounted for 81% of total proteins, with approximately 6% of the total represented by other proteins of higher molecular weight and 14% represented by other proteins and/or peptides of lower molecular weight. Therefore, on the basis that the NCF of 5.8 would reflect the nitrogen composition of a preparation containing thaumatin I and II proteins only and not the other proteins present in E 957 and that the Kjeldahl method does not differentiate between different proteins, the use of the general NCF of 6.2 (rounded from 6.25, (FAO, 2003)) is considered appropriate. The use of alternative analytical techniques for quantifying the total proteins in E 957 (e.g. HPLC-UV, HPLC-FLD, ELISA) was explored by an interested business operator and found not advantageous in terms of reducing the uncertainty of the protein estimates. This owing to the difficulty to obtain an analytical standard for thaumatin (E 957) with a purity sufficiently defined and consistent to be used as an analytical (calibration) standard; thus, the methods can be hardly validated.

The Panel noted that the definition of E 957 (currently reported under the chemical name in the EU specifications for E 957) should be amended in order to capture also the other proteins/peptides possibly present in the food additive in addition to thaumatin I and II. A more precise definition of E 957 can be '*Thaumatin is obtained by aqueous extraction (pH 2.5–4) of the arils of the fruit of Thaumatococcus daniellii (Benth) and consists essentially of the proteins thaumatin I and thaumatin II, together with other proteins and/or peptides, and minor amounts of plant constituents derived from the source material*'. The Panel also took note that the reported minimum purity assay reflects the total protein content in thaumatin (E 957), i.e. not less than 94% (established using Kjeldahl method with the current NCF of 6.2), whereas the actual content of thaumatin I and II proteins may be only four-fifths of this total, as indicated by one interested business operator. Therefore, the Panel considered that a new specification limit for the minimum combined content of thaumatin I and II proteins in E 957, determined by a validated analytical methodology for quantifying the thaumatin proteins, e.g. HPLC, should be introduced in the EU specifications for E 957.

Regarding the toxic elements, the Panel calculated the potential exposure to the toxic elements from the use of E 957 (see Appendix E). The resulting figures showed that the exposure to Pb and Al from the uses and use levels of E 957 would not be of concern using both the limit values calculated by the Panel as possible EU specification values (Table E.1 – Appendix E) and the existing EU specifications (Table E.2 – Appendix E). For As, its calculated MOS/MOE values (between 303 and 8,080, see Table E.2 Appendix E) indicate that a lowering of the existing limit value of 3 mg/kg is recommended. For Cd and Hg, for which no maximum limits are set in the EU specifications for E 957, the estimates of exposure are a very small fraction of their TWI values. The Panel noted that interested business operators indicated that the arils, from which thaumatin (E 957) is extracted, are not commercially cultivated but are harvested from the wild. So thaumatin (E 957) could be susceptible to contamination by toxic elements from environmental sources. Consequently, the Panel considered it appropriate to add limit values for Hg and Cd to the EU specifications alongside limit values for the three elements currently specified, namely Pb, As and Al (Table 1).

Because of its botanical origin, thaumatin (E 957) may be prone to microbiological contamination. The Panel noted that in addition to the already included specification limits for total aerobic microbial count and *E. coli*, further microbiological specifications for yeasts, moulds and *Salmonella* spp should be introduced on the basis of the data provided by the interested business operators. In addition to microbiological contamination, mycotoxins and pesticides residues may be possible contaminants in

E 957. Mycotoxins in the raw materials and commercial batches of E 957 were detected below quantifiable levels and the Panel considered that the ultrafiltration process performed alongside the purification steps should ensure that mycotoxin levels, if any, are reduced. Therefore, the Panel considered that there is no concern with respect to contamination by mycotoxins in E 957 and thus no need to introduce limit values for mycotoxins in the EU specifications of this food additive. Regarding pesticides, the analysis of one batch of thaumatin (E 957) for the presence of pesticides detected no residues. The Panel therefore considered that limit values for pesticides in the EU specifications of E 957 are not needed, as long as it is assured that arils are collected from plants that are not commercially cultivated. Should the food additive be prepared in full or in part from cultivated *T. daniellii*, limits for pesticides should be considered for inclusion in the EU specifications.

The Panel considered that thaumatin is a readily digestible protein. Acute toxicity studies in mice and rats showed no adverse effects up to 20 and 21 g/kg bw.

The Panel concluded that there was no concern with respect to genotoxicity for thaumatin (E 957).

No reproductive, chronic toxicity or carcinogenicity studies were available. In the sub-chronic studies in rats, no effects were observed on testis, uterus or ovaries.

Overall, the repeated dose toxicity studies and a prenatal developmental toxicity study in animals did not identify any adverse effects. Allergenicity via oral exposure was considered unlikely based on animal studies, but was possible via inhalation based on two observational studies in humans. Indications of allergenicity of thaumatin via inhalation in occupational settings are not considered relevant for dietary exposure.

Dietary exposure to thaumatin (E 957) was estimated according to different exposure scenarios based on consumers only as described in Section 3.4. Currently, thaumatin (E 957) is an authorised food additive in the EU in 15 food categories, while interested business operators provided EFSA with only one use level of thaumatin (E 957) in foods belonging to FC 11.4.3 Table-Top Sweeteners in tablets.

The highest mean and P95 exposure among consumers of one or more food categories containing thaumatin (E 957) was found in adolescents (0.08 and 0.33 mg/kg bw per day), while among consumers of individual food categories, the highest mean and P95 exposure was found in consumers only of FC 05.1 Chocolate and chocolate products in children with 0.17 and 0.48 mg/kg bw per day, respectively.

The Panel considered that the exposure to thaumatin (E 957) from its use as a food additive according to Annex II was overestimated in the regulatory maximum level exposure assessment scenario, given that exposure calculations based on the MPLs/maximum reported use levels were considered applicable to all foods within each food category, while the percentage of the foods in a subcategory labelled with thaumatin (E 957) in Mintel was maximally 3.2% (see Section 3.3.2).

In the refined brand-loyal scenario, the Panel considered the exposure to thaumatin (E 957) was underestimated due to the overall uncertainties (Table 5), and in particular that use levels were only available for table-top sweeteners, whereas thaumatin is labelled on other foods such as flavoured drinks (especially in energy drinks), food supplements as well as a small number of other subcategories in Mintel's GNPD.

Based on a rat 13-week NOAEL of 5,200 mg/kg bw per day (Ben-Dyke et al., 1976), the highest dose tested in males, including a factor of 2 for extrapolation from sub-chronic to chronic exposure (EFSA Scientific Committee, 2012a), a MOS of 5,417 was derived using the highest P95 exposure in consumers only (0.48 mg/kg bw per day based on the regulatory maximum level exposure assessment scenario). The Panel considered that the MOS is an underestimation since the exposure data are based on a regulatory maximum level exposure assessment scenario.

4.1. Uncertainty

The main sources of uncertainty relate to the absence of toxicological data on fertility, chronic toxicity and carcinogenicity. In addition, the few human intervention studies available were underpowered and associated with a high risk of bias. However, the fact that thaumatin is a readily digestible protein with no sign of adverse effects observed in any of the studies reviewed, including sub-chronic toxicity studies in rats and dogs, outweighs these limitations and reduces the overall uncertainty.

5. Conclusions

According to the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010 (EFSA ANS Panel, 2014) and given that:

- thaumatin is a digestible protein;
- adequate exposure estimates were available;
- there was no concern with respect to the genotoxicity;
- no conclusion on oral allergenicity could be drawn from the available human data
- no adverse effects were observed in sub-chronic toxicity studies in rats and dogs at the highest dose tested (5,200 mg/kg bw per day and 1,476 mg/kg bw per day, respectively) and in a prenatal developmental toxicity study (2,000 mg/kg bw per day);
- moderate confidence in the body of evidence supported the absence of association between exposure to thaumatin and adverse health outcomes

the Panel concluded that there is no need for a numerical ADI for thaumatin (E 957). Based on a calculated MOS of 5,417, considered to be an underestimate, the Panel concluded that there is no safety concern for thaumatin (E 957) at the regulatory maximum level exposure assessment scenario, which was considered the most appropriate.

6. Recommendations

The Panel recommends the European Commission to consider:

- introducing the CAS number 53850-34-3 in the EU specifications for E 957;
- introducing a new specification limit for the minimum combined content of thaumatin I and II proteins in E 957, determined by using a validated analytical methodology for quantifying specifically the thaumatin proteins, e.g. HPLC;
- lowering the existing EU maximum limit for arsenic and adding maximum limits for mercury and cadmium to the EU specifications for E 957;
- introducing a specification limit for yeast and mould counts and *Salmonella* spp in the EU specifications for E 957, based on the information provided by the interested business operators and Panel considerations.

7. Documentation provided to EFSA

1) Naturex SA, May 2018. Reply to the EFSA call for technical and toxicological data on sweeteners authorised as food additive in the EU (EFSA-Q-2017-00500). Technical data on thaumatin (E 957): section 1.
2) Naturex SA, September 2019. Reply to the EFSA call for technical data on sweeteners authorised as food additive in the EU (EFSA-Q-2019-00318) and reply to EFSA clarifications request on technical data provided in response to EFSA call for technical and toxicological data on sweeteners authorised as food additive in the EU (EFSA-Q-2017-00500).
3) Naturex SA, January 2021. Reply to EFSA clarifications request on technical data provided in response to EFSA calls for data on sweeteners authorised as food additive in the EU (EFSA-Q-2017-00500, EFSA-Q-2019-00318). Including the unpublished study report: Wageningen Food & Biobased Research, Report 2113, 2020. Digestibility study Thaumatin. Unpublished report.
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5) International Sweeteners Association (ISA), September 2019. Reply to the EFSA call for technical data on sweeteners authorised as food additive in the EU (EFSA-Q-2019-00318) and reply to EFSA clarifications request on technical data provided in response to EFSA call for technical and toxicological data on sweeteners authorised as food additive in the EU (EFSA-Q-2017-00500).
6) International Sweeteners Association (ISA), December 2020. Reply to EFSA clarifications request on technical data provided in response to EFSA calls for data on sweeteners authorised as food additive in the EU (EFSA-Q-2017-00500, EFSA-Q-2019-00318).

7) Naturex SA, May 2018. Reply to the EFSA call for technical and toxicological data on sweeteners authorised as food additive in the EU (EFSA-Q-2017-00500). Toxicological data on thaumatin (E 957), including unpublished study reports, section 2:

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Abbreviations

ADI	Acceptable Daily Intake
ADME	absorption, distribution, metabolism and excretion
Al	aluminium
ANS	EFSA Panel on Food Additives and Nutrient Sources added to Food
As	arsenic
BMDL	benchmark dose lower bound
bw	bodyweight
Cd	cadmium
cfu	colony-forming units
ELISA	enzyme-linked immunosorbent assay
FAF	EFSA Panel on Food Additives and Flavourings
FC	food category
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
GD	gestational days
GNPD	Global New Products Database
Hg	mercury

HPLC	high performance liquid chromatography
HPLC-UV	high-performance liquid chromatography with ultraviolet detection
HPLC-FLD	HPLC with fluorescence detection
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC-MS/MS	LC tandem mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MOE	Margin of exposure
MOS	Margin of Safety
MPL(s)	maximum permitted level(s)
NCF	nitrogen conversion factor
NOAEL	no observed adverse effect level
NTP	US National Toxicology Program
OES	optical emission spectroscopy
Pb	lead
P95	95th percentile
QS	*quantum satis*
RI	refractive index
RoB	Risk of bias
RP-HPLC	reversed phase HPLC
SCF	Scientific Committee on Food
SEM	scanning electron microscopy
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
TVC	Total viable count
TWI	tolerable weekly intake
VSSA	volume specific surface area
WoE	Weight of evidence

Appendix A – Tailored protocol for the assessment of hazard identification and characterisation applied for thaumatin (E 957)

Please refer to steps **1.1**–**1.15** of the general protocol for hazard identification and characterisation of sweeteners (EFSA, 2020a).

For step **1.11** (Extensive literature searches), the general principles reported in the protocol applied. The extensive literature searches were conducted in the three selected databases with the search strings and criteria applied as follow:

- **Web of Science**: TOPIC: (thaumatin OR E957 OR "E 957" OR "53850-34-3") AND LANGUAGE: (English) Timespan: 1988-2021.[24] Indexes: SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC.
- **Pubmed**: ((thaumatin OR E957 OR "53850-34-3") AND (("1988"[Date - Publication]: "2021[24]"[Date - Publication]))) AND ("english"[Language]) Quoted phrase not found: "E 957".
- **SciFinder**: Substance Identifier "53850-34-3 ">substances (1)>get references>refine "1988-2021[24]">refine "English">refine "Clinical Trial Journal Preprin…".

Additional data were submitted by the interested business operators through the call for biological and toxicological data on sweeteners.[6]

The final number of references that were screened, after removal of duplicates (based on title, year, author, journal, volume, issue and page numbers) and excluded document types (book, book section, conference proceedings), was 1519.

For step **1.11.1** (Screening of the studies for relevance), the general principles reported in the protocol applied. 155 papers were included at the level of title and abstract screening, whereas 1364 papers were excluded. From the 155 papers included for the full text screening, 18 were considered as possibly relevant, whereas 137 were either excluded at the level of full text screening, or preliminarily categorised into technical data (79), exposure data (20), environmental data (2) or toxicological review (5), and further screened for confirmation of their potential relevance (see Figure A.1 for PRISMA flow chart).

[24] Until May 2021.

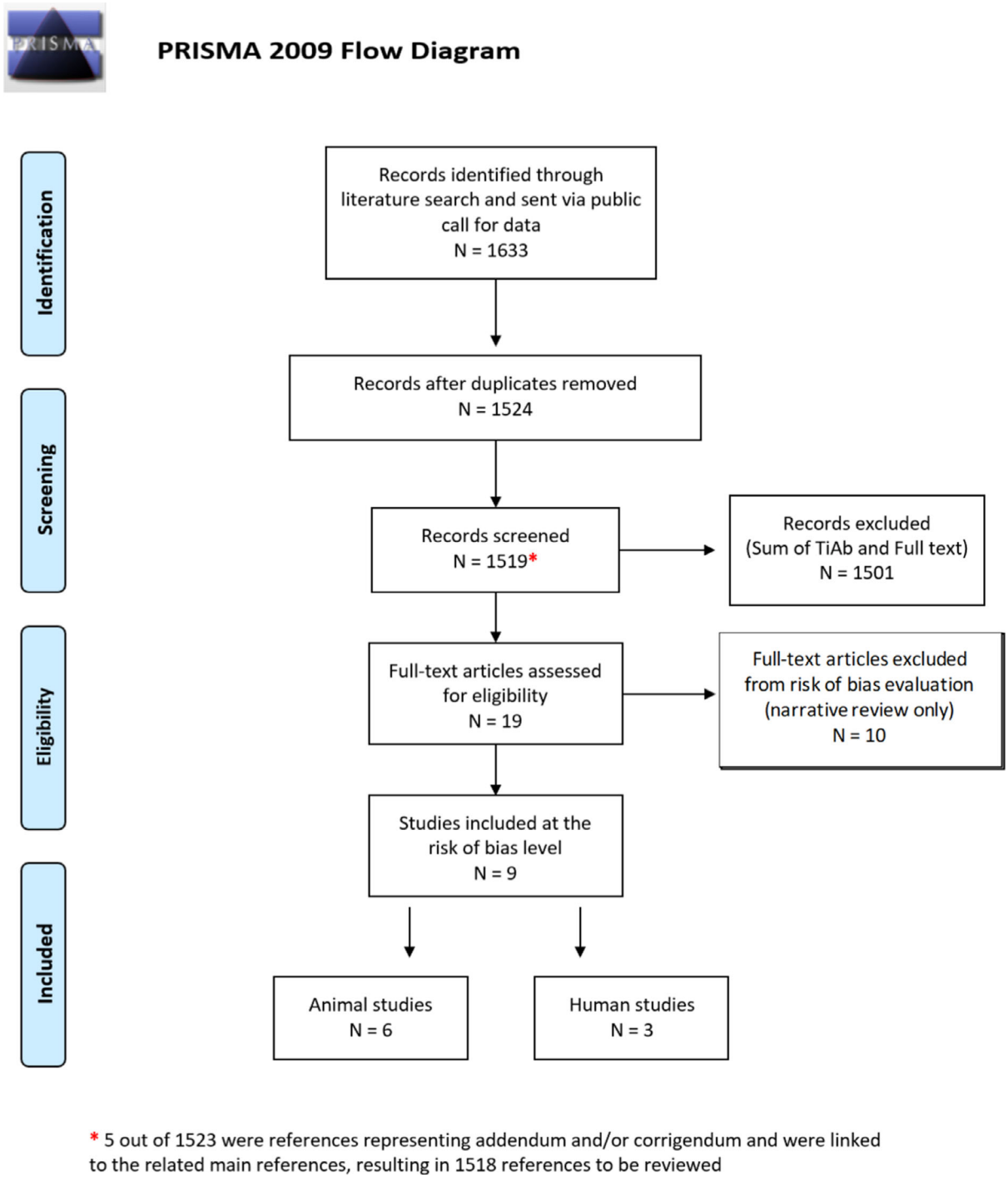

Figure A.1: PRISMA flow chart (adapted from Moher et al., 2009)

For step **1.12** (Evaluation of the risk of bias (RoB)), the criteria outlined in the protocol for the risk of bias evaluation of studies have been applied and the results of the RoB evaluation of the studies are summarised in Table A.1 for animal studies and in Table A.2 for human studies.

The study evaluated for the RoB was allocated to a Tier (from 1 to 3 corresponding to decreasing levels of internal validity) based on the rules as reported in step **1.12** (Evaluation of the risk of bias).

The evaluation of the RoB was conducted in parallel independently by two reviewers and in case a conflict on Tier allocation of a study was identified, ad hoc discussion between the reviewers took place prior to a final agreed Tier allocation that was reached by consensus.

The Tier allocation was based on the rules detailed in step **1.12** of the protocol, and was automatically generated by the DistillerSR tool, after input of the ratings for the individual elements of

the study considered for the risk of bias. At the end of the evaluation, the reviewers were requested to express their agreement or disagreement on the Tier generated by the tool based on their expert judgement. No disagreements were identified. In case of disagreement, a clear justification should have been provided (see last two columns on the right of Tables A.1 and A.2).

Table A.1: RoB of experimental animal studies

RefID	Authors and year	Study type	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Experimental conditions (performance bias	Blinding of research personnel (performance bias)	Incomplete outcome data (attrition bias)	Confidence in exposure characterisation (detection bias)	Confidence in outcome assessment (detection bias)	Selective reporting (reporting bias)	Appropriateness of statistical methods (other source of bias)	Tier (based on rules)	Tier (agreement with expert judgement)
1429	Danks et al. (1984)	Short-term toxicity	–	++	+	+	++	+	+	++	++	2	Yes
1430	Ben-Dyke et al. (1976)	Sub-chronic toxicity	++ / +	+	++	+	++	++ / +	++	++	+ / –	1	Yes
1425	Hiscox et al. (1981)	Sub-chronic toxicity	++ / +	+ / –	++	+	++	++ / +	++	++	+	1	Yes
1435	Hagiwara et al. (2005)	Sub-chronic toxicity	++	+	++	+	+	–	+	++	–	2	Yes
1433	Barker et al. (1981)	Sub-chronic toxicity	++	+	++	+	++	+	++	++	–	1	Yes
1420	Tesh et al. (1977b)	Developmental toxicity	+	+	++	+	++	–	+	–	+	2	Yes

Definitely low risk of bias (++), probably low risk of bias (+), probably high risk of bias (–), definitely high risk of bias (––).
Split cells reporting two different scorings for the same risk of bias question express the view of the two independent reviewers.

Table A.2: RoB of human studies

RefID	Authors and year	Study type	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Population for appropriate comparisons (selection bias)	Confounding and modifying variables (confounding bias)	Blinding of participants and personnel (performance bias)	Incomplete outcome data (attrition bias)	Confidence in exposure to the interventions (detection bias)	Confidence in outcome assessment (Detection bias)	Selective reporting (reporting bias)	Appropriateness of statistical methods (other source of bias)	Tier (based on rules)	Tier (agreement with expert judgement)
1419	Tompkins and	HCT	+	–	NA	NA	–	+	+	+	++	––	3	Yes

RefID	Authors and year	Study type	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Population for appropriate comparisons (selection bias)	Confounding and modifying variables (confounding bias)	Blinding of participants and personnel (performance bias)	Incomplete outcome data (attrition bias)	Confidence in exposure to the interventions (detection bias)	Confidence in outcome assessment (Detection bias)	Selective reporting (reporting bias)	Appropriateness of statistical methods (other source of bias)	Tier (based on rules)	Tier (agreement with expert judgement)
	Enticknap (1984)													
1428	Eaton et al. (1981)	HCT	+	–	NA	NA	–	++ +	+	+	++	––	3	Yes
1423	MacLeod et al. (1981)	HCT	+	–	NA	NA	–	+	+	+	–	––	3	Yes

Definitely low risk of bias (++), probably low risk of bias (+), probably high risk of bias (–), definitely high risk of bias (––), not applicable question for this type of study (NA).
Split cells reporting two different scorings for the same risk of bias question express the view of the two independent reviewers.

For step **1.13** (Data extraction), information and data from the included oral human intervention and toxicity animal studies as well as from the included genotoxicity studies were extracted and reported in tabular form in Appendix C and Appendix D of the opinion. The data extraction forms outlined in the draft protocol should be considered as templates and for this reason were slightly revised and adapted during the data extraction.

For step **1.14** (Weighing the body of evidence), a WoE analysis for different health outcome categories, grouped by endpoint as appropriate, was performed and presented in Excel tables in Annexes C and D. A narrative synthesis of the body of evidence (step **1.15**) was included in the opinion (see Section 3.5.4 Synthesis of systematically appraised evidence on biological and toxicological effects).

In principle, systematically appraised studies which were rated Tier 3 in the RoB evaluation should not be included in the WoE analysis (see Section 2.2 Methodologies). For thaumatin, the available animal studies included in the WoE analysis were all rated as Tier 1 and Tier 2. However, the three included human studies were rated as Tier 3, but because no other data were available, these were included in the WoE analysis.

Tier 1 and 2 animal studies were rated for initial confidence according to study design. The initial confidence in the body of evidence was downgraded for risk of bias, unexplained inconsistency, relevance of endpoints (in terms of adversity) and/or imprecision. Following the downgrading procedure, confidence could be upgraded for the following reasons: large magnitude of association or effect, evidence for a dose-response and consistency across species, study type or model. The human studies were downgraded for the same reasons as the animal studies, but could be upgraded for consideration of residual confounding instead of consistency across studies. For a more detailed description of assessment criteria, see NTP Handbook for conducting a literature-based health assessment using OHAT approach for systematic review and evidence integration OHAT (NTP-OHAT, 2019).

In accordance with the draft protocol for hazard identification and characterisation of sweeteners (EFSA 2020a), the confidence in the body of evidence was rated 'High', 'Moderate', 'Low' or 'Very low or No evidence identified' corresponding to the symbols '++++', '+++', '++', '+', respectively. Following the WoE analysis, confidence ratings were translated into levels of evidence for health effect or no health effect. The following descriptors were used to rate the level of evidence for the presence of

health effect: 'High', 'Moderate', 'Low' or 'Inadequate' when the confidence in the body of evidence was 'High', 'Moderate', 'Low' or 'Very Low or No evidence identified', respectively. In cases where no health effect was present, a modified version of the draft protocol[25] (EFSA, 2020a) was used. The corresponding descriptors were: 'Evidence of no health effect' when the confidence in the evidence was 'High', 'Moderate' when the confidence in the evidence was 'Moderate', and 'Inadequate' when the confidence in the evidence was either 'Low' and 'Very low or No evidence identified' (Figure A.2, adapted from NTP-OHAT, 2019).

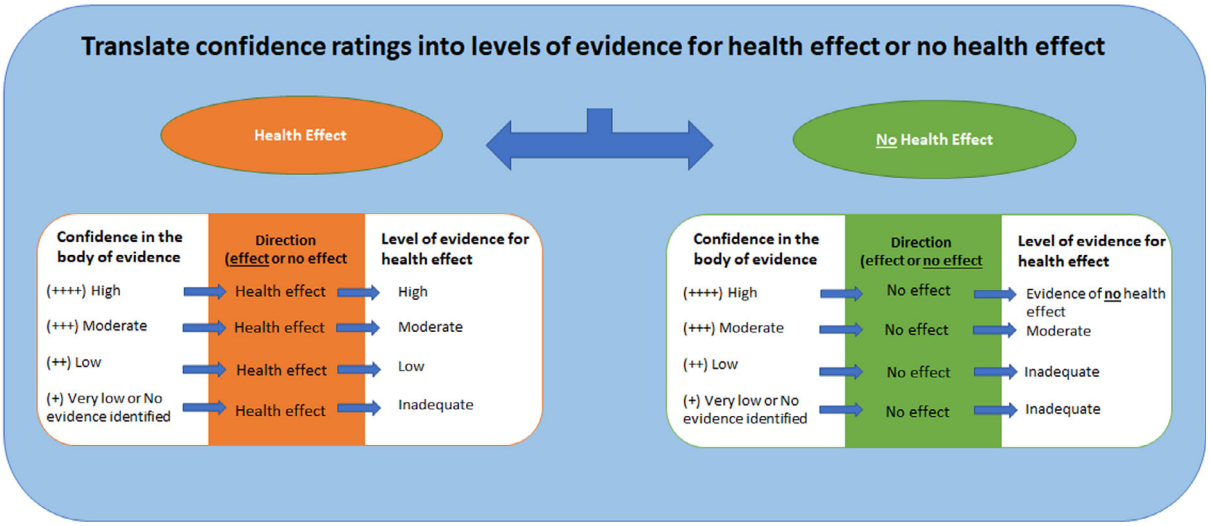

Figure A.2: Translation of confidence ratings into evidence of health effect conclusions (adapted from NTP-OHAT, 2019)

[25] From the draft protocol for hazard identification and characterisation of sweeteners (EFSA 2020a): 'When the overall confidence in the evidence is high (++++) for an endpoint where the effect is absent, the absence of an effect will be considered 'highly likely'. Furthermore, if the evidence is scored less than ++++, the level of evidence of a health effect will be considered inadequate'.

Appendix B – Biological and toxicological data

RefID	Source	Author(s)	Title	Year
1316	Literature	Tschannen MP, Gluck U, Bircher AJ, Heijnen I, Pletscher C	Thaumatin and gum arabic allergy in chewing gum factory workers	2017
1419	Call for data	Tompkins GD, Enticknap JB	A Comparison of the Effects on the Chemical and Cellular Composition of Blood Following the Administration of Thaumatin and Egg Albumin to Human Subjects for 13 Weeks	1984
1420	Call for data	Tesch JM, Earthy M, Tesch SA, Willoughby CR	Talin: Effects of Oral Administration upon Pregnancy in the Rat	1977
1421	Call for data	Tesch JM, Davidson EJ, Willoughby CR	Talin: Test for Dominant Lethality in the Male Mouse	1977
1422	Call for data	Stanworth DR	Preliminary Assessment of the Potential Allergenicity of the Sweet Protein, Talin	1977
1423	Call for data	MacLeod GL, Eaton KK, Daniel JW, Snodin DJ, Higginbotham JD, Waite D	Talin Protein: Assessment of Oral Sensitisation and Irritation when Formulated in Peppermint Chewing Gum	1981
1424	Call for data	Higginbotham JD	Mutagenicity Testing of Talin Protein Sweetener in Vitro	1980
1425	Call for data	Hiscox DN, Hill RE, Wood CM	Talin Protein: 90 Day Toxicity Study in the Rat by Dietary Admixture	1981
1426	Call for data	Edwards DG	Talin (Thaumatin): Nitrogen Digestibility in the Rat	1981
1427	Call for data	Higginbotham JD	The Digestibility of Talin Protein in Vitro	1978
1428	Call for data	Eaton KK, Daniel JW, Snodin DJ, Higginbotham JD, Stanworth DR, Al-Mosawie T	Talin Protein: Assessment in Man for Oral Allergenicity on Challenge Testing	1981
1429	Call for data	Danks A, Hooks W, Ashby R, Whitney JC	Talin: Effect on Thyroid Function in Rats Following Dietary Administration - Final Report	1984
1430	Call for data	Ben-Dyke R, Ashby R, Newman, AJ	Talin: Toxicity in Dietary Administration to Rats for Thirteen Weeks	1976
1431	Call for data	Ben-Dyke R	Talin: Acute Oral Toxicity in Mice	1975
1432	Call for data	Ben-Dyke R, Joseph EC	Talin: Acute Oral Toxicity in Rats	1976
1433	Call for data	Barker JD, Hiscox DN, Wood CM	Talin Protein: 90 Day Toxicity Study in the Dog by Dietary Admixture	1981
1435	Literature	Hagiwara A, Yoshino H, Sano M, Kawabe M, Tamano S, Sakaue K, Nakamura M, Tada M, Imaida K, Shirai T	Thirteen-week feeding study of thaumatin (a natural proteinaceous sweetener), sterilized by electron beam irradiation, in Sprague-Dawley rats	2005
1445	Literature	Higginbotham, JD, Snodin DJ, Eaton KK, Daniel JW	Safety evaluation of Thaumatin (talin protein)	1983
1916	Reply to EFSA clarifications request	Wageningen Food & Biobased Research	Digestibility study Thaumatin	2020

Appendix C – Data extraction forms for toxicological studies

Studies that are described in this Appendix are only reflecting the information provided in the study reports/papers.

Short-term toxicity studies

Study ID	
RefID (DistillerSR)	**1429**
Reference (authors, year, title, other info)	Danks A, Hooks W, Ashby R and Whitney JC, 1984. Talin: Effect on Thyroid Function in Rats Following Dietary Administration - Final Report. Report No. 84/TYL073/601. Life Science Research. Eye, Suffolk, UK.
Source (published/unpublished)	Unpublished
Type of study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)[26]	No
Type of study[27]	Short-term toxicity
Animal model	
Species and strain	Rats, CD strain
Disease models (e.g. diabetes, allergy, obesity)	Not applicable
Housing conditions	
Housing condition	Rat were housed in polypropylene cages. Five rats of one sex held per cage. Water was available from the public supple and a complete low fat powdered rodent diet was available ad libitum before and during the treatment period.
Diet name and source (if reported)	Pelleted rodent diet, Charles River formula
Treatment	
Test material[28]	Talin
Provider	Sponsor
Compound purity	Talin analysis provided (Nitrogen 16.54%, Total Ash (sulfated) < 0.1%, Carbohydrate 0.96%, Water 5.14%)
Vehicle used	None (direct admixture with powder rodent diet)
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available[29]	0%, 3% w/w in the diet Control group was administered with 3% of egg albumin. Equivalent to: 3600 mg/kg bw per day
Route of administration (diet, drinking water, gavage)	Diet
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	4 weeks

[26] i.e. use of EPA, OECD, FDA or other guideline for study design.
[27] e.g. acute, short-term (i.e. up to 28 days, 4 weeks), sub-chronic (i.e. up to 90 days, 13 weeks), chronic (i.e. up to 2 years, 104 weeks), reproduction/developmental, carcinogenicity.
[28] As reported by the authors. In the case of thaumatin, the trade name Talin was commonly used and it was used to indicate the proteins extract derived from the arils of the fruits of strains of *Thaumatococcus daniellii (*Benth).
[29] Based on analytical data and measured feed (water) intake (μg, mg or g/kg bw per day, mean for a whole study period, when the test compound is given in the diet (water)). In gavage studies 'achieved doses' are based on analytical data for a concentration of a test compound. When the doses were not explicitly reported by the authors as mg/kg bw per day and was not possible to be calculated from the analytical data and measured feed (water) intake, default factors were applied (see 2.2 Methodologies) and equivalence to mg/kg bw per day was reported.

Study design	
Sex and age at the start of the treatment	Males and females rats (26–33 days)
Number animals/sex per group	10/males per group; 10/females per group For thyroid weight (right lobe and left lobe): only 9/ females per group
Measured endpoints[30]	Clinical signs, bodyweight, food consumption, water intake, T3 and T4 levels (plasma), macroscopic necropsy, adrenals weight, pituitary weight, thyroid weight (absolute and relative)
Time of measurement/observation period[31]	Rats and cages were inspected daily for evidence of reaction to treatment or ill-health. Bodyweight was recorded at the beginning of the study and weekly thereafter. The quantity of food consumed by each cage was calculated weekly after correcting for the residues in the food hoppers and the amount scattered. T3 and T4 level in the plasma, absolute and relative organ weights, gross pathology at the end of treatment
Methods to measure the endpoints[32]	Thyroid was weighed with the parathyroids, organ tissues were preserved in buffered 4% formaldehyde saline, blood samples were withdrawn from the retro-orbital sinus, with the animal held under light ether anaesthesia
Statistical analysis	
Statistical methods	Student's t-test, Dunnett's test
Results	
Findings reported by the study author/s	Talin had no effect on bodyweight and there was no evidence of any reaction to treatment. The amount of food consumed by treated males was comparable to that of the control group whereas it was reduced some 7% in the females. The levels of thyroxine and tri-iodothyronine in the plasma at the end of the study were similar for the test and control groups. Treatment was without effect on thyroid, pituitary and adrenals weights and no macroscopic changes that could be attributed to Talin were recorded. It was concluded that the administration of Talin to CD rats at a dietary concentration of 3% for 4 weeks was without effect on thyroid function and adrenal weight.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound[33]	Not applicable
Further information	
No	

[30] In case of guideline studies, state only if there were any deviation from the guideline (e.g. missing and/or additional endpoints).
[31] For reproductive and developmental toxicity studies, please indicate the life stage at which the measurement/observations were done i.e. pre-mating, mating, gestation, lactation, adult). For short-term, sub-chronic, chronic and carcinogenicity study it should be indicated in which week of the study or if at the beginning or the end of the treatment measurements/ observations were done. In case of guideline studies, state only if there were any deviation from the guideline.
[32] Particularly relevant for new or non-standard methods and/or for endpoints not covered by test guidelines. In case of guideline studies, state only if there were any deviation from the guideline.
[33] If and as reported by the study author.

Sub-chronic toxicity studies

Study ID	
RefID (DistillerSR)	1430
Reference (authors, year, title, other info)	Ben-Dyke R, Ashby R and Newman AJ, 1976. Talin: Toxicity in Dietary Administration to Rats for Thirteen Weeks. Report No. 76/TYL4/188. Life Science Research. Stock, Essex, UK.
Source (published/unpublished)	Unpublished
Type of study and guideline	
Good laboratory practice (yes/no/No, but before establishment of GLP)	No
Guideline studies (if yes, specify)[26]	No
Type of study[27]	Sub-chronic toxicity
Animal model	
Species and strain	Rats, CD strain
Disease models (e.g. diabetes, allergy, obesity)	Not applicable
Housing conditions	
Housing condition	The rats were accommodated in suspended polypropylene cages, with stainless steel mesh floors and lids. Five rats were held in each cage. Ambient temperature was maintained at 21 ± 2°C. Lighting was controlled to provide 12 h of light and 12 h of dark in each 24 h.
Diet name and source (if reported)	Powdered rodent diet (Spratts Laboratory Animal Diet No. 2)
Treatment	
Test material[28]	Talin, sweet principle of *Thaumatococcus daniellii*
Provider	Sponsor
Compound purity	82% protein (carbohydrates 2.2%, ash 3.5%, water 6.6%)
Vehicle used	None (direct admixture with powder rodent diet)
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available[29]	0, 1, 4, 8% w/w in the diet Control group received casein (8% w/w in the diet) Equal to: 1%: 696 mg/kg bw per day in males; 889 mg/kg bw per day in females 4%: 2,608 mg/kg bw per day in males; 3,350 mg/kg bw per day in females 8%: 5,200 mg/kg bw per day in males; 6,608 mg/kg bw per day in females
Route of administration (diet, drinking water, gavage)	Diet
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	13 weeks
Study design	
Sex and age at the start of the treatment	Males and females (7 weeks)
Number animals/sex per group	10/males per group; 10/females per group For haematology: 10/males and 10/females only for the control group and the high-dose group For clinical chemistry and urinalysis: 5/males per group; 5/females per group For liver and kidney function: 5/males per group; 5/females per group For histopathology: 5/males and 5/females for the control group; 10/males and 10/females for the high-dose group (apart for mammary gland for which 10/males per group and 10/females per group were examined).

Measured endpoints[30]	Clinical signs, mortality, bodyweight, food consumption, water intake, haematology, clinical chemistry, urinalysis, 6-h urine concentration test, bromsulfthalein (BPS) clearance, serum antibody titre, gross pathology, organ weights and histopathology
Time of measurement/observation period[31]	Cages were inspected once daily for mortality and clinical signs, during the first 10 days of the treatment period this visual examination was made two or three times daily. Food consumption was calculated by each cage at weekly intervals, water intake was subjected to a visual observation daily. Bodyweight was recorded at the beginning of the study and subsequently at weekly intervals. Blood samples were withdrawn at week 12 for the control and high-dose group for assessing haematological parameters and clinical chemistry (the latter for 5 males and 5 females of all treated groups). Urine samples were collected at week 12, using metabolic cages. For the urine concentration test, urine samples were collected at week 12. BPS clearance was measured at week 12 in 5 males and 5 females of the control and high dose group. For the serum antibody titre, blood was collected at week 13 for 5 male and 5 female animals in all groups.
Methods to measure the endpoints[32]	In line with standard sub-chronic toxicity study. For serum antibody titre: blood was obtained by cardiac puncture. The samples were allowed to clot and the separated serum was analysed for antibodies to Talin, using Ouchterlony diffusion technique.
Statistical analysis	
Statistical methods	Student's t-test
Results	
Findings reported by the study author/s	No death occurred. The bodyweight gain of male rats fed with 4 and 8% w/w Talin in the diet was 6% and 9% lower than that of the casein-fed controls. This reduction assumed statistical significance at weeks 11, 12 and 13 in animals at the high-dose group. The bodyweight gain of female rats was not affected by treatment. The food consumption of all male rats and females receiving 4 and 8% w/w Talin in the diet was some 5–11% lower than that of the casein-fed controls. The cellular and chemical composition of blood and urine was not affected by treatment. Although there was a statistically significant reduction in the haemoglobin content of the female rats fed 8% Talin, the values were within the range normally found for animals of this age and weight. Tests for hepatic and renal function, in rats fed 8% Talin revealed responses that were similar to those obtained in the casein-control group. No evidence was obtained for the presence in the serum of treated animals of antibodies to Talin. Necropsy revealed no treatment-related macroscopic pathology. There was a statistically significant increase in both absolute and relative liver weight of female rats receiving 8% Talin. No such effect was apparent among the males in this group or in female rats of the mid-dose and low-dose groups. There were isolated deviations from control values, some of which assumed statistical significance, in the absolute and relative weight of heart, spleen and thyroids, among animals at the lower dose-levels. The differences were not appreciable and there was no dosage-related trends to suggest that these represented a response to treatment. A wide range of tissues was examined

	microscopically, all of which were devoid of any morphological change that could be attributed to treatment.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound[33]	Not reported
Further information	
In the control, casein was supplemented in the basal laboratory diet in order to compensate for the high protein intake. Analysis of compound were not reported. Composition of the samples used in the study are stated to be protein (82%), carbohydrate (2.2%), ash (3.5%), water (6.6%).	

Study ID	
RefID (DistillerSR)	1425
Reference (authors, year, title, other info)	Hiscox DN, Hill RE and Wood CM, 1981. Talin Protein: 90 Day Toxicity Study in the Rat by Dietary Admixture. Report Ref. TAL/1/81. Toxicol Laboratories Ltd. Ledbury, UK.
Source (published/unpublished)	Unpublished
Type of study and guideline	
Good laboratory practice (yes/no/No, but before establishment of GLP)	GLP regulations as set forth in the U.S. Code of Federal Regulations Title 21, part 58 (1978)
Guideline studies (if yes, specify)[26]	No
Type of study[27]	Sub-chronic toxicity
Animal model	
Species and strain	Rats, CD strain
Disease models (e.g. diabetes, allergy, obesity)	Not applicable
Housing conditions	
Housing condition	Rats were randomly allocated to polypropylene cages. Five rats of one sex were housed per cage. Temperature at 21°C +/− 2, relative humidity 60 +/− 10%. Lighting was controlled to provide 14 h light and 10 h dark in each 24 h cycle. All animals received complete powdered rodent diet and bottled mains water ad libitum throughout the treatment period.
Diet name and source (if reported)	Powdered rodent diet (B.P. Nutrition, Witham, Essex, England)
Treatment	
Test material[28]	Talin protein sweetener
Provider	Sponsor
Compound purity	Talin analysis provided (Nitrogen 15.65%, Total Ash (sulfated) 0.3%, Carbohydrate 1.5%, Water 5.4%). Batch was also analysed after the completion of the study (Nitrogen 15.99%, Total Ash (sulfated) 0.6%, Carbohydrate Not determined, Water 5.35%)
Vehicle used	None (direct admixture with powder rodent diet)
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available[29]	0, 0.3, 1, 3% w/w in the diet Control group received untreated diet Equal to: 0.3%: 256 mg/kg bw per day in males; 293 mg/kg bw per day in females 1%: 748 mg/kg bw per day in males; 998 mg/kg bw per day in females, 3%: 2,418 mg/kg bw per day in males; 2,822 mg/kg bw per day in females

Route of administration (diet, drinking water, gavage)	Diet
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	90 days
Study design	
Sex and age at the start of the treatment	Males and females (4–5 weeks)
Number animals/sex/group	20/males per group; 20/females per group For haematology, clinical chemistry and urinalysis: 10/males per group; 10/females per group
Measured endpoints[30]	Almost in line with the OECD TG 408 (no measurement of T4, T3 and TSH levels) Clinical signs and survival rate, body weight, food consumption, water intake, ophthalmology, urinalysis, haematology, clinical chemistry, gross pathology, organs weights, histopathology
Time of measurement/observation period[31]	All animals were checked for reaction daily for reaction to treatment. Bodyweight was recorded at the beginning of the study and then at weekly intervals until termination. Food consumption was recorded by each cage of rats and the group mean weekly intake was calculated. Water was visually inspected daily throughout the treatment period. Individual cage water intakes were recorded over a period of three consecutive days, once during the acclimatisation period and during week 6 and 13. Ophthalmological investigations were performed before the beginning of treatment and in the control and high dose group during week 12. Urine and blood samples collected during week 4 and 12 from 10 males and females of each group.
Methods to measure the endpoints[32]	In line with standard sub-chronic toxicity study
Statistical analysis	
Statistical methods	Student's t-test
Results	
Findings reported by the study author/s	There were no visible signs of reaction to treatment. No animals died as a result of treatment with Talin protein. Five animals died following blood sampling during the treatment period. Bodyweight values for males receiving 3% w/w Talin protein were slightly higher than the control values and females receiving 1% showed a slight reduction compared to controls from week 6 onwards. All remaining groups were unaffected. Food consumption values for females receiving 3% were slightly lower than control. Values for remaining groups was considered normal. No difference in water intake was detected. Ophthalmoscopic examination revealed no abnormalities that could be attributed with Talin. Various changes in haematology were not considered to be consistent with the administration of Talin. Urinalysis was unaffected by treatment. No treatment related changes were seen during macroscopic examination. Changes in absolute and body weight related thyroid weights in treated animals compared with controls were not considered to be of toxicological significance. No changes were seen microscopically that were considered to be related to the administration of Talin.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound[33]	Not reported

Further information
An addendum to this study is available: Wood C.M (1984) Talin Protein: 90 Day Toxicity Study in the Rat by Dietary Admixture - Further Microscopic Examination of Thyroids. Report Ref. TAL/1/C. Toxicol Laboratories Ltd. Ledbury, UK. This study reported a more detailed microscopic assessment of thyroid gland histopathology in order to try and pinpoint any changes which might correlate with the statistically significant group and sex variations in thyroid gland weights. A single section of the right and left thyroid gland stained with H and E was examined from all male and females in groups 1–4. Microscopic observations were reported for all these animals. Minimal colloid distension of follicles was seen in a proportion of glands form all groups. The extent of this minor change ranged from minimal distension of occasional follicles and minimal distension of a proportion of follicles to minimal generalised follicular distension in occasional animals and moderate colloid follicular distension in rat 43 and 104. However, there was no group or treatment related variation and this minor change was considered to be within normal limits. Occasional very small papillary projections of epithelium into distended follicles were seen in a few animals in both control and treatment groups but was not considered to be significant. Minor changes in thyroid follicular size was within the normal range. In conclusion no changes were seen in the thyroid sections examined which were considered to be related to the administration of the test compound Talin. One batch was used also in RefID 1433 (Barker et al., 1981, 90-day dog study).

Study ID	
RefID (DistillerSR)	1433
Reference (authors, year, title, other info)	Barker JD, Hiscox DN and Wood CM, 1981. Talin Protein: 90 Day Toxicity Study in the Dog by Dietary Admixture. Report Ref. TAL/2/81. Toxicol Laboratories Ltd. Ledbury, UK.
Source (published/unpublished)	Unpublished
Type of study and guideline	
Good laboratory practice (yes/no/no, but before establishment of GLP)	In compliance with the GLP regulations as set forth in the U.S. Code of Federal regulations Title 21, part 58 (1978)
Guideline studies (if yes, specify)[26]	No
Type of study[27]	Sub-chronic toxicity
Animal model	
Species and strain	Dog, beagle
Disease models (e.g. diabetes, allergy, obesity)	Not applicable
Housing conditions	
Housing condition	Dogs were allocated into singles galvanised pens with a solid concrete floor with dimensions of 150 × 75 cm. All dogs received a total of 400 g/day of diet and food was presented in metal bowls. At all times, except during urine sampling, the dogs were allowed free access to filtered mains water via automatic drinking valves. The room was illuminated by fluorescent light to give an artificial 24 h cycle of 14 h light/ 10 h dark. The room was air-conditioned with a system designed to maintain the air temperature at 16 ± 2°C
Diet name and source (if reported)	Lab A diet expanded, ground (B.P. Nutrition (UK) Limited, Witham, England)
Treatment	
Test material[28]	Talin protein sweetener, First batch was used up to week 11 (included), second batch was used from week 12 until the end of the study.
Provider	Sponsor
Compound purity	First batch (Nitrogen 15.65%, total ash (sulfated) 0.3%, carbohydrate 1.5%, water 5.4%) Second batch (Nitrogen 16.5%, total ash (sulfated) 0.12%, carbohydrate 0.9%, water 3.8%) The two batches were also analysed after the completion of the study:

	First batch (Nitrogen 15.99%, Total ash (sulfated) 0.6%, carbohydrate ND, water 5.35%) Second batch (Nitrogen 16.0%, Total ash (sulfated) 0.18%, Carbohydrate 1.6%, Water 5.3%)
Vehicle used	None (direct admixture with diet)
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available[29]	0, 0.3, 1.0, 3.0% w/w in the diet Equal to: 0.3%: 133 mg/kg bw per day in males; 139 mg/kg bw per day in females 1.0%: 435 mg/kg bw per day in males; 469 mg/kg bw per day in females 3.0%: 1,305 mg/kg bw per day in males; 1,476 mg/kg bw per day in females
Route of administration (diet, drinking water, gavage)	Diet
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	90 days
Study design	
Sex and age at the start of the treatment	Males and females (5 months)
Number animals/sex per group	4/males per group; 4/females per group
Measured endpoints[30]	Clinical signs, bodyweight, food consumption and water intake, ophthalmoscopy, haematology, clinical chemistry, urinalysis, gross pathology, organ weights, histopathology.
Time of measurement/observation period[31]	Body weight was recorded weekly and feed intake daily. Water intake was measured over three consecutive days before the start and in weeks 6 and 13 of the study. Ophthalmoscopic examination was conducted before the start and in week 12 of the study. Blood samples and urine were collected before the start, in weeks 4 and 12 of the study.
Methods to measure the endpoints[32]	In line with standard sub-chronic toxicity study.
Statistical analysis	
Statistical methods	Student's t-test
Results	
Findings reported by the study author/s	No deaths occurred during the treatment period. There were no overt signs of reaction to treatment. Males receiving Talin protein showed slightly increased bodyweight relative to controls. The group mean bodyweights of all female groups remained similar to controls throughout the study. Food consumption was unaffected by treatment. Water intake was unaffected by treatment. Ophthalmoscopic examination of the eyes did not reveal any changes that could be related to treatment. Haematological examination of blood samples revealed decreases in haemoglobin concentration, erythrocyte count and packed cell volume in males of 3% w/w during weeks 4 and 12 of treatment. Biochemical examination did not reveal any treatment-related effects. Urinalysis was unaffected by treatment. Macroscopic pathology at termination did not reveal any treatment-related changes. An increase in absolute liver weight was noted in males 3% w/w group, and of kidneys in 0.3% w/W group. A decrease in the weight of the spleen of females at 0.3% w/w group was observed. Bodyweight-related organ weights showed no treatment-related variations. No changes were seen in the sections examined microscopically that were considered to be related to the administration of the compound under

	test. It is considered that the minor effects observed were of doubtful biological significance.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound[33]	Not reported
Further information	
The choice of treatment levels was based on the results of a palatability study which showed 3% w/w Talin protein in the diet to be palatable to one male and one female dog over 7 days (data not available), and on the fact that similar treatment levels were employed in a 90-day study in the rat. One batch was used also in RefID 1425 (Hiscox et al., 1981, 90-day rat study).	

Study ID	
RefID (DistillerSR)	1435
Reference (authors, year, title, other info)	Hagiwara A, Yoshino H, Sano M, Kawabe M, Tamano S, Sakaue K, Nakamura M, Tada M, Imaida K and Shirai T, 2005. Thirteen-week feeding study of thaumatin (a natural proteinaceous sweetener), sterilized by electron beam irradiation, in Sprague-Dawley rats. *Food and Chemical Toxicology*, 43, 1297–1302. https://doi.org/10.1016/j.fct.2005.04.001
Source (published/unpublished)	Published
Type of study and guideline	
Good laboratory practice (yes/no/no, but before establishment of GLP)	Conducted in compliance with the Good Laboratory Practice (GLP) Standards of the Japanese Ministry of Health and Welfare Ordinance NO.21 (March 26th 1997). Analyses regarding the prepared diets and test substance were not performed under GLP conditions.
Guideline studies (if yes, specify)[26]	Guidelines for Designation of Food Additives and for Revision of Standards for Use of Food Additives of the Japanese Ministry of Health and Welfare (Eika No.29, March 22nd, 1996). In house guideline for the Care and Use of Laboratory Animals. This protocol is comparable to the recommended in the OECD TG 408.
Type of study[27]	Sub-chronic toxicity
Animal model	
Species and strain	Rat, Crj:CD (SD) IGS rats
Disease models (e.g. diabetes, allergy, obesity)	Not applicable
Housing conditions	
Housing condition	Animal were housed in transparent polypropylene cage on hardwood chip bedding in an environment-controlled room. Constant temperature 22 +/– 2 'C, humidity 55 +/– 10% and ventilation more than 15 times/h were maintained, and the room was artificially illuminated for 12 h daily.
Diet name and source (if reported)	Certified powdered diet Labo MR stock (Nihon Nosan Kogyo K.K., Yokohama)
Treatment	
Test material[28]	Thaumatin irradiated Lot no. J89. Thaumatin non-irradiated Lot No. 981028.
Provider	Talin Food Company (Merseyside, UK)
Compound purity	101%
Vehicle used	None (direct admixture with powder rodent diet)

Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available[29]	Irradiated thaumatin: 0, 0.3, 1.0 and 3.0% Non-irradiated thaumatin: 3.0% Equal to: Irradiated thaumatin 0.3%: 260 mg/kg bw per day in males; 299 mg/kg bw per day in females 1.0%: 788 mg/kg bw per day in males; 1,042 mg/kg bw per day in females 3.0%: 2,502 mg/kg bw per day in males; 2,889 mg/kg bw per day in females Non-irradiated thaumatin 3.0%: 2,394 mg/kg bw per day in males; 2,925 mg/kg bw per day in females
Route of administration (diet, drinking water, gavage)	Diet
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	13 weeks
Study design	
Sex and age at the start of the treatment	Males and females (5 weeks old)
Number animals/sex/group	10/males per group; 10/females per group For histopathology: 10/males and 10/females for control and high dose groups only For urinalysis: 6/males per group; 6/females per group
Measured endpoints[30]	Almost in line with the OECD TG 408 (no measurement of T4, T3 and TSH levels) Clinical signs and survival rate, body weight, food consumption, water intake, urinalysis, ophthalmology, haematology, clinical chemistry, gross pathology, organs weights, histopathological examination of tissues.
Time of measurement/observation period[31]	Almost in line with the OECD TG 408. The animals were observed daily for abnormalities, and individual body weights were recorded weekly. Food consumption was measured over a 2-day period before each weighing. An ophthalmologic examination was performed on all animal groups during week 13. Urinalysis of samples collected over 4 h (from 9:00 AM to 1:00 PM) was conducted for 6 animals in each sex/group during week 13. Blood samples were collected at week 13 after sacrifice.
Methods to measure the endpoints[32]	Semiquantitative estimation (Multistix, Miles-Sankyo Co., Ltd., Tokyo) of protein, glucose, ketones, bilirubin, occult blood and urobilinogen. Specific gravity values were measured using a reflectance meter (Atago Co., Ltd., Tokyo, Japan). The levels of urinary electrolytes (sodium, potassium and chlorine) were determined using a Hitachi Biochemical Automatic Analyser, model 7040E (Hitachi Co., Ltd, Tokyo, Japan). Haematological estimations were carried out using an automatic blood cell counter, model F-820 (Sysmex Co., Kobe, Japan) and using a Dry Haematosystem COAG1 (A&T Co., Tokyo, Japan). Differential counts of leukocytes and reticulocyte counts were made by microscopy of specimens, stained, respectively, with Wright-Giemsa and Brecher. Blood biochemistry determinations were performed with a Hitachi-Biochemical Automatic Analyser, model 7040E (Hitachi Co., Ltd., Tokyo, Japan).

Statistical analysis	
Statistical methods	For body weight, urinalysis, haematology, blood biochemistry and organ weight data, the significance of intergroup differences was assessed using the two-tailed Student's t-test. Insufficient homogeneity of variance was corrected with respect to the degrees of freedom according to Welch (1947). For the incidences of non-neoplastic and neoplastic lesions, the significance of differences observed between the control and treated groups was evaluated with the Fisher's exact probability test. The levels of significance were set at $P < 0.05$ and 0.01
Results	
Findings reported by the study author/s	No clinical signs or deaths related to irradiated thaumatin treatment were observed. A tendency for lower body weights were noted in both sexes of rats receiving 1.0 and 3.0% irradiated thaumatin. No body weight retardation was noted in rats fed 3.0% non-irradiated thaumatin. Food consumption in controls and treated animals showed no clear differences. Food efficiency in the treated groups was the same as in the controls. Water consumption in the treated groups was similar to the control level throughout the study. No treatment-related adverse effects were noted in any group on urinalysis. No ophthalmological changes were found in any animals of the control and treated groups. No treatment-related adverse effects were apparent from the haematology results or in any parameters of blood biochemistry. No treatment-related macroscopic changes were found in treated animals at autopsy or on detailed gross examination after fixation. No treatment-related changes in organ weights and organ to body weight ratios were observed in either sex of the highest dose (irradiated and non-irradiated) groups. No treatment-related histopathological changes were observed in either sex of the 3.0% irradiated and non-irradiated thaumatin groups. The present results indicate that electron beam irradiated thaumatin does not exert any adverse effects on any of the parameters examine here. Therefore, the toxicologic hazard of actual intake (2mg/person; which is equivalent to 0.03 mg/kg for an adult of 60 kg body weight) of irradiated thaumatin, estimated from thaumatin under normal conditions can be considered negligible.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound[33]	NOAEL: 3% (2502 mg/kg bw per day for males; 2,889 mg/kg bw per day for females)
Further information	
Percentage % content was calculated from the nitrogen content x factor 6.25; molecular weight was approximately 22,000; characteristics as powder with a particular flavour. The specification of irradiated and non-irradiated thaumatin were the same. For bodyweight, only a graph available, no tabulated results (mean or individual values). Histopathological examinations only for the control and highest dose groups. Data of urinalysis, haematology, clinical chemistry, macroscopic and microscopic findings, organ weights were not shown in the paper.	

Developmental toxicity study

Study ID	
RefID (DistillerSR)	1420

Reference (authors, year, title, other info)	Tesh JM, Earthy M, Tesh SA and Willoughby CR, 1977b. Talin: Effects of Oral Administration upon Pregnancy in the Rat. Report No. 77/TYL10/179. Life Science Research. Stock, Essex, UK.
Source (published/unpublished)	Unpublished
Type of study and guideline	
Good laboratory practice (yes/no/no, but before establishment of GLP)	No
Guideline studies (if yes, specify)[26]	No
Type of study[27]	Developmental toxicity
Animal model	
Species and strain	Rat, CD strain
Disease models (e.g. diabetes, allergy, obesity)	Not applicable
Housing conditions	
Housing condition	The rats were held in high density polypropylene cages. Room temperature was controlled at 21 ± 2°C, humidity at 50 ± 10% RH, and a 12 h light: 12 h dark cycle was in operation.
Diet name and source (if reported)	Pelleted rodent diet (Lab. Diet No 1 manufactured by Spratts Patent Ltd)
Treatment	
Test material[28]	Talin
Provider	Sponsor
Compound purity	Not reported
Vehicle used	Distilled water
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available[29]	0, 200, 600, 2,000 mg/kg bw per day
Route of administration (diet, drinking water, gavage)	Gavage
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Gestation
Duration of the exposure	Gestation day (GD) 6–15
Study design	
Sex and age at the start of the treatment	Females (not reported)
Number animals/sex/group	20 females/group
Measured endpoints[30]	Maternal clinical signs, dam bodyweight, dam gross pathology, number of pregnant animals, number of corpora lutea, number of implantations, number of viable fetuses, resorptions, pre-implantation loss, post-implantation loss, litter size fetal weight, fetal external, visceral and skeletal abnormalities
Time of measurement/observation period[31]	All animals were observed daily throughout the study. Bodyweights were recorded on days 1, 6, 8, 10, 12, 14, 15, 18 and 21 of gestation. At termination, rats were sacrificed and examined macroscopically for evidence of disease or adverse reaction to treatment and the reproductive tract was dissected and examined.
Methods to measure the endpoints[32]	In line with standard developmental toxicity study
Statistical analysis	
Statistical methods	Not reported
Results	
Findings reported by the study author/s	Appearance and general condition of Talin-treated rats remained indistinguishable from those of control animals. Bodyweight gain of all treated groups was similar to that of the control group. At examination post mortem, no

	evidence was found of any treatment-related macroscopic change in maternal tissues. Mean live litter size was comparable with control for all treated groups as well as mean foetal weight. One female in the top dosage group (2,000 mg/kg bw per day) displayed total litter loss, but in this respect other females in this group were comparable to controls and therefore not considered treatment-related. Overall, implantation losses were identical in all groups. A small number of abnormalities were found in foetuses in treated and control groups. These were of a type found to occur spontaneously in this strain of rat and their incidence showed no treatment-related association or trend. In conclusion, oral administration of Talin to pregnant rats from Day 6 to Day 15 of gestation at levels of up to 2.0 g/kg per day, was without adverse effect upon the pregnant dam or upon the developing fetus.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound[33]	NOAEL: 2,000 mg/kg bw per day for maternal and developmental effects
Further information	
Only information available for the tested material: a 500 g sample of Talin (mix No 9) was received as a pale brown, slightly granular powder. No certificates of analysis available in the study report. Endpoints measured in line with OECD 414.	

Human studies

Study ID	
RefID (DistillerSR)	1419
Reference (authors, year, title, other info)	Tompkins GD and Enticknap JB, 1984. A Comparison of the Effects on the Chemical and Cellular Composition of Blood Following the Administration of Thaumatin and Egg Albumin to Human Subjects for 13 Weeks.
Source (published/unpublished)	Unpublished
Study design	
Study type[34]	HCT
Type of blinding	Single-blinded
Duration of the study and length of follow-up	13 weeks
Subjects	
Number of participants in the study	30 participants
Number of exposed/non-exposed subjects or number of cases/controls (if applicable)	15 exposed; 15 non-exposed
Sex (male/female)	9 males; 6 females per group
Age (mean or range as reported)	19–55 years old
Geography (country)	UK
Ethnicity	Not reported
Confounders and other variables as reported	No
Special health condition of participants	Not reported
Inclusion and exclusion criteria in the study	Not reported
Other information	Volunteers were employees of a thaumatin production facility
Intervention/exposure	
Test material	Thaumatin (Nitrogen 17.7%, Total ash (sulfated) 0.24%, Carbohydrates not determined, Water 6.22%)

[34] e.g human controlled trial (HCT), cohort study (Co), case-control study (CaCo), cross-sectional study (CrSe).

Description of the intervention and estimated dietary exposure	Capsules, daily ingestion (9.00 AM). Each capsule contained 280 mg Thaumatin or 210 mg Egg albumin
Co-exposure description (if applicable)	Not applicable
Endpoint measured, measurement time points and methods	Haematology, clinical chemistry at week 0, 4, 8, 12. Haemoglobin, white blood cells, red blood cells, platelets, mean corpuscular volume, packed cell volume, mean corpuscular haemoglobin concentration and erythrocyte sedimentation rate. Sodium, potassium, bicarbonate, urea, glucose, creatinine, bilirubin (total), protein, albumin, globulin (by difference), cholesterol, triglycerides, high- and low-density lipoprotein, uric acid, alkaline phosphatase, aspartate transaminase and y-glutamyl transferase.
Were subgroups analyses predefined? (yes/no, including justification)	Not applicable
Results	
Findings reported by the study author/s	The value for y-glutamyltransferase (gamma-GT) was abnormal for 2 subjects. Haemoglobin, red cell count and mean corpuscular volume were abnormal in 2 other subjects and another subject had abnormal bilirubin. None of the abnormalities were related to treatment. Each parameter is within the physiological range and there were no classifiable variations that could be attributed to the ingestion of thaumatin. The cumulative intake of thaumatin by subjects 1–15 in the present study was 25 g which is some 140 times the estimated maximum consumer intake over this period. The absence of any systematic effect on the composition of the blood and of any subjective reaction among the individual participants provides further evidence for the innocuous nature of the product.
Statistical analysis	
Statistical methods (including power analyses, multiple comparison, potential sources of bias, adjustment for confounders, test for interactions):	Not performed (only mean and SD reported)
Further information	
Formal t-testing was not performed; however, a consultant pathologist reviewed the data by visual inspection. Review of the data is available in the study report.	

Study ID	
RefID (DistillerSR)	1423
Reference (authors, year, title, other info)	MacLeod GL, Eaton KK, Daniel JW, Snodin DJ, Higginbotham JD and Waite D, 1981. Talin Protein: Assessment of Oral Sensitisation and Irritation when Formulated in Peppermint Chewing Gum.
Source (published/unpublished)	Unpublished
Study design	
Study type[34]	HCT
Type of blinding	Double-blinded
Duration of the study and length of follow-up	28 days; within 2 days after completion of 28-day period
Subjects	
Number of participants in the study	50 participants
Number of exposed/non-exposed subjects or number of cases/controls (if applicable)	25 exposed; 25 non-exposed

Sex (male/female)	Exposed: 17 males; 8 females Non-exposed: 19 males; 6 females
Age (mean or range as reported)	17–61 years old
Geography (country)	UK
Ethnicity	Not reported
Confounders and other variables as reported	One volunteers within the exposed group had already a small benign papilloma on his tongue before starting the study.
Special health condition of participants	Not reported
Inclusion and exclusion criteria in the study	Not reported
Other information	Volunteers were employees of a thaumatin production facility
Intervention/exposure	
Test material	Talin Protein Sweetener (Nitrogen 15.74%, Total ash (sulfated) 0.3%, Carbohydrates not determined, Water 7.6%)
Description of the intervention and estimated dietary exposure	Double mint gum containing 150 ppm thaumatin. Each subject chewed five 5 gum sticks per day (5.3 g each) for 15 min for period of 28 days. The other group received untreated control gum.
Co-exposure description (if applicable)	Not applicable
Endpoint measured, measurement time points and methods	Weal and flare responses to histamine, oral mucosa examination. Skin prick test on initiation of the study and within 2 days following the study termination, visual examination of oral cavity before and after the study. Prick test: using serial dilution of talin of 10,000, 5,000, 2,500 and 1,000 protein nitrogen units (p.n.u.), passed the test for sterility adopted by the European Pharmacopoeia Commission.
Were sub-groups analyses predefined? (yes/no, including justification)	No subgroup analyses
Results	
Findings reported by the study author/s	There was no evidence of any oral allergic or irritant response to the test material when administered in the chewing gum over a period of 28 days.
Statistical analysis	
Statistical methods (including power analyses, multiple comparison, potential sources of bias, adjustment for confounders, test for interactions):	Not performed
Further information	
Within the exposed group: one volunteer was withdrawn because of flu after 14 days; one had already a small benign papilloma on his tongue, which started to sore for the first time but this participant was not excluded from the study.	

Study ID	
RefID (DistillerSR)	1428
Reference (authors, year, title, other info)	Eaton KK, Daniel JW, Snodin DJ, Higginbotham JD, Stanworth DR and Al-Mosawie T, 1981. Talin Protein: Assessment in Man for Oral Allergenicity on Challenge Testing.
Source (published/unpublished)	Unpublished
Study design	
Study type[34]	HCT

Type of blinding	Double-blinded
Duration of the study and length of follow-up	14-day (follow by a cross-over period of further 14 days)
Subjects	
Number of participants in the study	10 participants
Number of exposed/non-exposed subjects or number of cases/controls (if applicable)	5 exposed; 5 non-exposed
Sex (male/female)	6 males; 4 females
Age (mean or range as reported)	Not reported
Geography (country)	UK
Ethnicity	Not reported
Confounders and other variables as reported	Possible fracturing of the capsule could permit detection of the sweet taste of the substance (instruction were given to swallow the capsule directly to avoid it). Three subjects included in the study have tasted Talin previously over a 5 year period in gram quantities. One subject had an exquisite ability to identify taste of Talin protein (taste Talin trials previously undertaken), she identified Talin during the study and therefore withdrawn. One subject was involved in weighing Talin as a dry powder.
Special health condition of participants	Two subjects had pre-existing facial dermatitis. One subject worked with acetone and ether and as a result suffered dry skin.
Inclusion and exclusion criteria in the study	Not reported
Other information	Volunteers were employees of a thaumatin production facility
Intervention/exposure	
Test material	Talin Protein Sweetener (Nitrogen 14.14%, Total ash (sulfated) 0.48%, Carbohydrates not determined, Water 6.56%)
Description of the intervention and estimated dietary exposure	Gelatin capsules, daily ingestion. Each capsule contained 100 mg Talin protein or 100 mg Lactose.
Co-exposure description (if applicable)	Not applicable
Endpoint measured, measurement time points and methods	Weal and flare responses, blood samples Skin prick test on initiation of the study and shortly after the study termination. Prick test: using serial dilution of talin of 10,000, 5,000, 2,500 and 1,000 protein nitrogen units (p.n.u.), passed the test for sterility adopted by the European Pharmacopoeia Commission. Blood samples collected at the beginning of the study and at the end: used for Passive Cutaneous Anaphylaxis (PCA) test (in monkeys)
Were sub-groups analyses predefined? (yes/no, including justification)	No, but a patch test on one subject was performed during the course of the study because of facial contact dermatitis.
Results	
Findings reported by the study author/s	None of the subjects developed positive weal or flare to Talin. All were tested in two occasions. None of the 9 subjects who completed the study showed evidence of sensitisation either by skin testing or by symptom scores. The P.C.A. titres for all subjects were also negative. The patch test performed during the study to one subject, that showed active facial contact dermatitis, was considered to be negative indicating an absence of cutaneous sensitisation.

	The study did not show any evidence of oral allergenicity of Talin using a total per capita dosage of 1.4 gram for 14 days which probably represents a years intake for an average consumer.
Statistical analysis	
Statistical methods (including power analyses, multiple comparison, potential sources of bias, adjustment for confounders, test for interactions):	Not performed
Further information	
For the withdrawn subject, prick tests and PCA were, nevertheless, completed and were negative. For the PCA test in monkeys, a baboon and a rhesus monkey was used. Serum from the study subjects was intradermally injected into the abdomen of the animals, together with a negative (serum from not allergic subject) and positive (serum from grass pollen-sensitive subject) control. Each monkey was then injected intravenously with 2% Eva's blue followed by 10 mg Talin (1 ml) by the same route. As a result, no change in skin colour occurred, indicating a negative PCA reactivity, while positive control revealed an intense blue reaction.	

Appendix D – Data extraction forms for genotoxicity studies

Study ID (DistillerSR)	1424
Reference (authors, year, title, other info)	Higginbotham JD. Mutagenicity testing of Talin protein sweetener in vitro. Unpublished report from Tate & Lyle PLC, dated 20 August 1980
Funding	Private
Good laboratory practice (GLP) compliance and guideline	Good laboratory practice: no Guideline studies: not available at the date of the study
Test system	Reverse mutation in *Salmonella* Typhimurium TA1535, TA1537, TA1538, TA98, TA100 and *Escherichia coli* WP2, with and without metabolic activation
Test material	Talin protein, composition unspecified, dissolved in distilled water
Exposure/treatment conditions	Preincubation method. Doses: from 50 to 50,000 μg/plate, with one plate per dose
Results	Negative. No treatment related increase in revertant colonies was observed, with or without metabolic activation. The report also included another set of test results performed in a different laboratory with the same set of Salmonella strains and *E. coli* WP2 uvrA (no other details provided). Also this study reported negative results in the dose range evaluated (50–30,000 ug/plate, one plate per dose).
Other comments	The study, performed before the development of the relevant OECD Guideline, followed a very limited protocol, with single platings instead of triplicate as recommended in OECD Guideline. This is a major deviation from currently agreed criteria. The reliability of the study is inadequate and its relevance for genotoxic hazard identification is low.

Study ID (DistillerSR)	1421
Reference (authors, year, title, other info)	Tesh JM et al. Test for dominant lethality in the male mouse. Unpublished report from Tate & Lyle PLC, dated 4 April 1977a
Funding	Private
Good laboratory practice (GLP) compliance and guideline	Good laboratory practice: no Guideline studies: not available at the date of the study
Test system	Dominant lethal assay in male CD-1 mice.
Test material	Talin protein, composition unspecified, dissolved in distilled water
Exposure/treatment conditions	Male CD-1 mice (15 per group) were administered by gavage at 200 and 2,000 mg/kg bw for five consecutive days. Trimethyl phosphate (100 mg/kg bw) served as positive control. At the end of treatment each male was mated with 3 females, which were sacrificed 4, 11 and 18 days post-coitum. Mating procedure was repeated for 7 weeks.
Results	Negative. Inspection of the uterine content did not show any effect of thaumatin administration on fertilisation, pre- and post-implantation losses.
Other comments	The study was performed before the development of the relevant OECD Guideline but the protocol is considered scientifically acceptable. This non-GLP study is evaluated as reliable with restriction, and of limited relevance because the end-point addressed is not recommended as first tier for genotoxic hazard identification.

Appendix E – Exposure calculations to toxic elements from the use of thaumatin as a food additive

The interested business operators provided analytical data on the levels of lead (Pb), arsenic (As), mercury (Hg), cadmium (Cd) and aluminium (Al) in commercial batches of thaumatin (E 957).

One interested business operator provided results for nine batches of E 957 and for all 5 elements the results were reported as being < 0.1 mg/kg, with this being the Limit of Quantification (LOQ) for the method used (Documentation provided to EFSA nr: 4, 6).

The other interested business operator initially provided data for four batches of E 957 on the levels of Pb, Cd, As and Hg determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and for seven batches of E 957 on the levels of Al determined by optical emission spectroscopy (OES) (Documentation provided to EFSA nr: 1, 2). Upon clarifications request, the interested business operator provided analytical data on levels of Pb, Cd, As and Hg in a further five batches of E 957 by ICP-MS analysis. In summary, from this interested business operator, Pb (9 batches) was in the range < 0.04 to 0.058 mg/kg. As (nine batches) was in the range 0.03–0.29 mg/kg. Hg (seven batches) was all < 0.005 mg/kg. Cd (seven batches) were all ≤ 0.011 mg/kg. Al (seven batches), one batch was < 0.5 and the other 6 batches were in the range of 17–35 mg/kg (Documentation provided to EFSA nr: 2, 3).

Based on the analytical data provided, Hg and Cd were not detected above the LOQ of 0.1 mg/kg. Making an allowance to account for representativeness, homogeneity and analytical measurement uncertainty, limit values for these two elements could be set at three times the LOQ values, giving Hg = 0.3 and Cd = 0.3 mg/kg. For the elements that were detected, setting target values at approximately three times the highest level reported for the batches analysed gives Pb = 0.3, As = 1 and Al = 100 mg/kg.

The potential exposure to toxic elements from the use of the food additive E 957 can be calculated by assuming contamination of the additive may be up to the limit values 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, the Panel considered values (Table 4, Section 3.4.1) of: (i) Regulatory maximum level exposure assessment scenario; Mean up to 0.08 and P95 up to 0.33 mg/kg bw per d; (ii) Refined brand-loyal exposure assessment scenario; Mean up to 0.01 and P95 up to 0.02 mg/kg bw per d. The above mentioned 'modulated' maximum limits combined with the estimated intakes of thaumatin (E 957) could result in an exposure which can be compared with the following reference points or health based guidance values for the five elements; a $BMDL_{01}$ of 0.3–8 μg/kg bw per day for arsenic (EFSA CONTAM Panel, 2009), a $BMDL_{01}$ of 0.5 μg/kg bw per day for lead (EFSA CONTAM Panel, 2010), a TWI of 2.5 μg/kg bw per day for cadmium (EFSA CONTAM Panel 2011), a TWI of 4 μg/kg bw for mercury (EFSA CONTAM Panel, 2012) and a TWI of 1 mg/kg bw for aluminium (EFSA AFC Panel, 2008).

The outcome of such an exercise (Table E.1) illustrates the health impact that could result if the calculated maximum limits for toxic elements were to be used.

Table E.1: Risk assessment for toxic elements based on the analytical data submitted by interested business operators, and 'modulated' by the Panel, in thaumatin (E 957)

Exposure to E 957 (mg/kg bw per day)[a]	MOS/MOE for As at 1 mg/kg	MOS/MOE for Pb at 0.3 mg/kg	% of the TWI for Cd at 0.3 mg/kg	% of the TWI for Hg at 0.3 mg/kg	% of the TWI for Al at 100 mg/kg
0.33[b]	910–24,200	5,050	0.03	0.02	0.02
0.02[c]	15,000–400,000	83,333	< 0.01	< 0.01	< 0.01

(a): Data from Table 4 (Section 3.4.1).
(b): Regulatory maximum level exposure assessment scenario; Mean < 0.01–0.08; P95 < 0.01–0.33 mg/kg bw per day.
(c): Refined brand-loyal exposure assessment scenario; Mean < 0.01–0.01; P95 < 0.01–0.02 mg/kg bw per day.

Since Pb, As and Al already have limit values in the specifications of E 957, Table E.2 illustrates the health impact that could result if the existing EU specification limits for these 3 toxic elements were maintained.

Table E.2: Risk assessment for toxic elements based on the existing EU specifications for thaumatin (E 957)

Exposure to E 957 (mg/kg bw per day)[(a)]	MOS/MOE for As at 3 mg/kg	MOS/MOE for Pb at 3 mg/kg	% of the TWI for Al at 100 mg/kg
0.33[(b)]	303–8,080	505	0.02
0.02[(c)]	5,000–133,333	8,333	< 0.01

(a): Data from Table 4 (Section 3.4.1).
(b): Regulatory maximum level exposure assessment scenario; Mean < 0.01–0.08; P95 < 0.01–0.33 mg/kg bw per day.
(c): Refined brand-loyal exposure assessment scenario; Mean < 0.01–0.01; P95 < 0.01–0.02 mg/kg bw per day.

For As the reference point is based on carcinogenicity for which the MOS/MOE should be at least 10,000 (EFSA, 2005; EFSA Scientific Committee, 2012b) and the MOS/MOE can be lower than this value (Tables E.1 and E.2) even though the Panel noted that the BMDL for As (EFSA CONTAM Panel, 2009) is derived from human studies and an interspecies extrapolation factor may not be needed. The assessment of the uncertainty in the exposure showed a potential for overestimation of exposure for both scenarios used (see Section 3.4.2, Table 6). Also, the actual content of contaminants in E 957 would be lower on average than the limit values used for the calculations. Despite that the MOS/MOE is likely to be underestimated by (i) the exposure estimates being conservative and by, (ii) using the limit values for arsenic, the calculated MOS/MOE values for arsenic indicate that a lowering of the existing limit value for As from 3 mg/kg is recommended.

For Pb 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. In the opinion on lead (EFSA CONTAM Panel, 2010) it is 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 $BMDL_{01}$ (MOS/MOE lower than 1). The MOS/MOE values for Pb are well above 1.

For Cd, Hg and Al the estimates of exposure are only a very small fraction of their TWI values.

Annex A – Exposure data and estimates

Dietary surveys used for the estimation of chronic dietary exposure to thaumatin (E 957).

Concentration data used in the exposure assessment scenarios (mg/kg or mL/kg as appropriate).

Number and percentage of food products labelled with food additive thaumatin (E 957) out of the total number of food products present in the Mintel GNPD per food subcategory between 2015 and 2020.

Summary of estimated exposure to thaumatin (E 957) for the maximum level exposure scenario and the refined exposure scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day).

Main food categories contributing to the exposure of thaumatin (E 957) (number of surveys by contribution class) in the MPL scenario.

Summary of estimated exposure to thaumatin (E957) for consumers only populations of each food, MPL and refined scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day).

Annex A can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2021.6884

Annex B – List of excluded studies

List of studies that did not meet the inclusion criteria established in the draft protocol on hazard identification and characterisation of sweeteners (EFSA, 2020a).

Annex B can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2021.6884

Annex C – Weight of Evidence (WoE) tables for animal studies

Annex C can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2021.6884

Annex D – Weight of Evidence (WoE) table for human studies

Annex D can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2021.6884