

ADOPTED: 13 December 2022

doi: 10.2903/j.efsa.2023.7775

## Re-evaluation of locust bean gum (E 410) as a food additive in foods for infants below 16 weeks of age and follow-up of its re-evaluation as a food additive for uses in foods for all population groups

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### Abstract

Locust bean gum (E 410) was re-evaluated in 2017 by the former EFSA Panel on Food Additives and Nutrient sources added to Food (ANS). As a follow-up to that assessment, the Panel on Food Additives and Flavourings (FAF) was requested to assess the safety of locust bean gum (E 410) for its uses as a food additive in food for infants below 16 weeks of age belonging to food category 13.1.5.1 (Dietary foods for infants for special medical purposes and special formulae for infants). In addition, the FAF Panel was requested to address the issues already identified during the re-evaluation of the food additive when used in food for the general population, including the safety assessment for FC 13.1.5.1 and 13.1.5.2 (Dietary foods for babies and young children for special medical purposes as defined in directive 1999/21/EC). The process involved the publication of a call for data. Based on the received data, the Panel concluded that the technical data provided by the interested business operators support an amendment of the specifications for locust bean gum (E 410) laid down in Commission Regulation (EU) No 231/2012. The Panel identified a reference point of 1,400 mg/kg bw per day based on reduced blood zinc levels in a piglet study. It applied the margin of exposure (MoE) for the safety assessment of locust bean gum (E 410) when used as a food additive in FC 13.1.5.1 and 13.1.5.2. The Panel concluded that a MoE above 1 would not raise a safety concern. A MoE above 1 was obtained for some of the scenarios and exposure levels for infants. For toddlers (consumers only of food for special medical purposes), the MoE was above 1 for all exposure levels.

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**Keywords:** locust bean gum, E 410, food additive, infants

**Requestor:** European Commission

**Question number:** EFSA-Q-2018-00095

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**Declarations of interest:** If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact [interestmanagement@efsa.europa.eu](mailto:interestmanagement@efsa.europa.eu).

**Acknowledgements:** EFSA wishes to thank the following for the support provided to this scientific output: Christina Kyrkou; the members of the FAF WG Specifications of Food Additives.

**Suggested citation:** EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes M, Aquilina G, Castle L, Degen G, Engel K-H, Fowler PJ, Frutos Fernandez MJ, Fürst P, Gürtler R, Husøy T, Manco M, Mennes W, Moldeus P, Passamonti S, Shah R, Waalkens-Berendsen I, Wright M, Wölflle D, Dusemund B, Mortensen A, Turck D, Barmaz S, Mech A, Rincon AM, Tard A, Vianello G and Gundert-Remy U, 2022. Re-evaluation of locust bean gum (E 410) as a food additive in foods for infants below 16 weeks of age and follow-up of its re-evaluation as a food additive for uses in foods for all population groups. *EFSA Journal* 2023;21(2):7775, 70 pp. <https://doi.org/10.2903/j.efsa.2023.7775>

**ISSN:** 1831-4732

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



## Summary

In accordance with Regulation (EU) No 257/2010, the European Food Safety Authority (EFSA) is currently re-evaluating the safety of food additives already permitted in the Union before 20 January 2009 and issuing scientific opinions on their safety when used in food as per Annexes II and III to Regulation (EC) No 1333/2008. The risk assessment approach followed in the re-evaluation has not covered the use of food additives in food for infants below 12 weeks of age. Additionally, while re-evaluating the safety of food additives referred to above, EFSA identified some concerns, namely (1) Data gaps that have triggered recommendations in the published scientific opinions; and/or; (2) Data gaps that have increased uncertainties linked to the risk assessment and/or which prevented the Panel from concluding on some aspects of it.

On 31 May 2017, EFSA published a guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age, thus enabling EFSA to assess the safety of food additives used in food for infants below this age. The age up to 16 weeks was selected in the guidance because infants are exposed to formula feeding until this age as the only source of food since complementary feeding is not supposed to be introduced before.

As follow-up of the above, this Opinion addresses the data gaps previously identified during the re-evaluation of locust bean gum (LBG; E 410) as a food additive and the safety in the special sub-population of infants below 16 weeks of age.

The process followed involved the publication of a dedicated call for data allowing all interested business operators to provide the requested information for completing the assessment and to confirm that the additive is present in special formulae for infants below 16 weeks of age under special medical conditions (FC 13.1.5.1). The data submitted in response to the call for data on LBG (E 410) comprised technical data, a study on neonatal piglets, clinical studies, post-marketing surveillance reports and literature studies.

According to Commission Regulation (EU) No. 231/2012, LBG (E 410) is defined as the ground endosperm of the seeds of the strains of carob tree, *Ceratonia siliqua* (L.) Taub. (family Leguminosae). It consists mainly of a high molecular weight hydrocolloidal polysaccharide, composed of galactopyranose and mannopyranose units combined through glycosidic linkages, which may be described chemically as galactomannan. LBG is generally used as a food additive due to its thickening and stabilising properties. The substance has the CAS Registry Number 9000-40-2 and the EINECS number 232-541-5. Specifications for LBG (E 410) have been defined in Commission Regulation (EU) No 231/2012.

Based on the data on particle size distribution submitted by the interested business operators (IBOs) and the criteria set in the relevant EFSA Scientific Committee Guidance, the Panel concluded that the presence of small particles, including nanoparticles, cannot be confirmed or excluded in the pristine food additive. The Panel noted that currently no standardised methods are available to measure the particle size distribution for the polysaccharide thickening and gelling agents used as food additives and that further research for method development is needed. The Panel noted, however, that polysaccharide thickening, and gelling agents used as food additives, to exert their technical function in general swell in liquid environments. This also applies to LBG (E 410). The FAF Panel considers that LBG will not be present in the gastrointestinal (GI)-tract in the pristine form taking into account the capacity to absorb and swell in water, and the volume of fluid in the stomach and GI tract.

In response to the call for data, analytical data on levels of four toxic elements (Pb, Hg, Cd and As) in commercial samples of LBG (E 410) (clarified and unclarified) were provided by IBOs. The Panel agreed that the lowest technologically achievable levels proposed by the IBOs for these toxic elements submitted for clarified LBG were consistent with the occurrence of data. However, the lowest technologically achievable levels, as well as the specifications proposed by the IBOs were much higher than most of the data reported for these four toxic elements in unclarified LBG.

The Panel considered the refined brand-loyal exposure scenario to calculate the exposure to the toxic elements from the use of E 410. For the general population, the highest mean and the highest 95th percentile in the refined brand-loyal exposure scenario were 237 and 435 mg/kg body weight (bw) per day in toddlers (EFSA ANS Panel, 2017). For infants below 16 weeks of age, the refined exposure estimates based on the maximum and mean use levels of E 410 reported by industry were considered. The mean and 95th percentile exposure estimates were 2,000 and 2,600 mg/kg bw per day for the maximum use level and 839 and 1,130 mg/kg bw per day for the mean use level reported. The highest mean and 95th percentile exposure for the population above 16 weeks of age and

toddlers (consumers only of food for special medical purposes (FSMP)) were calculated in this opinion as 1,107 and 1,653 mg/kg bw per day for infants above 16 weeks of age up to 1 year.

The Panel performed the risk assessment that would result if Pb, Hg, Cd and As were present in E 410 at (i) the maximum current limit in the EU specification; (ii) the lowest technologically achievable levels proposed by the IBOs for unclarified LBG (E 410); (iii) the lowest technologically achievable levels proposed by the IBOs for clarified LBG (E 410).

The Panel also calculated the impact of the potential level of the toxic elements lead and cadmium in the food additive (i.e. up to the specifications limit values) on the final product and compared that with the legal limits for these elements in the final formula for infants below 16 weeks of age set by Reg. (EC) No 1881/2006. Considering the results of these calculations and the fact that the food additive is not the only potential source of toxic elements in the infant formula the Panel emphasises the need to reduce the specification limit values for lead and cadmium in Regulation (EU) no 231/2012.

Consequently, the Panel concluded that the potential exposure to toxic elements resulting from the exposure to E 410 could be substantial.

The Panel noted that the maximum limits in the EU specifications for toxic elements should be established based on actual levels in the commercial food additive. Therefore, the Panel recommended to lower the maximum limits on the basis of the information provided by the IBO and on the considerations of the Panel. The Panel does not see the need to introduce separate specifications for toxic elements in unclarified and clarified LBG (E 410).

In response to the call for data the IBOs provided analytical data on levels of residual proteins, total aerobic microbial count (TAMC), total combined yeast and mould count (TYMC), *E. coli*, *Salmonella* spp. and *Cronobacter (Enterobacter) sakazakii* in both clarified and unclarified LBG (E 410) preparations. The levels of TAMC and TYMC reported for clarified LBG were lower than the one reported for the unclarified LBG. All three IBOs reported data on *E. coli* and *Salmonella* spp. which were not detected in any commercial sample and only one IBO submitted data for *Cronobacter (Enterobacter) sakazakii* for commercial batches of unclarified LBG, for which the results were negative (neg/10 g). The Panel noted that E 410 may be prone to the microbiological contamination and therefore microbiological specifications should be set in the EU specifications. Although one IBO claimed that the process of clarification of LBG reduces the microbial load, and the limited data provided seem to support this, the Panel does not see the need to introduce separate microbiological specifications for unclarified and clarified LBG (E 410).

The Panel noted that the levels of residual protein in commercial samples of unclarified and clarified LBG differ. Residual protein in commercial samples of unclarified LBG ranged up to 7% as reported by for two IBOs, which is the current EC specification limit. For clarified LBG, one IBO reported protein levels up to 0.5% and they proposed a separate specification of not more than 1% of protein in clarified LBG. Although clarified LBG contains a lower level of residual proteins than unclarified gum, the Panel does not see the need to introduce separate specifications for residual protein in unclarified and clarified LBG (E 410) because no indication for differences in adverse reactions are observed in clinical studies or newly submitted post-marketing surveillance data where only one case of allergic reaction has been reported. Regarding the case reports only two studies reporting adverse events for two infants were provided.

Taking all these aspects into consideration, the Panel has made proposals for an update of the EC specifications for LBG (E 410).

Dietary exposure to LBG (E 410) for infants below 16 weeks of age from its use as a food additive was assessed based on (1) maximum permitted levels (MPLs) set out in the EU legislation (defined as the regulatory maximum level exposure assessment scenario) and (2) the reported use levels (defined as the refined exposure assessment scenario). Both scenarios are based on the recommended consumption levels from the relevant Scientific Committee Guidance which recommends values of 200 and 260 mL formula/kg bw per day as conservative mean and high-level consumption values for 14- to 27-day-old infants.

For infants below 16 weeks of age consuming special infant formulae (FC 13.1.5.1), exposure to LBG (E 410) in the regulatory maximum level exposure assessment scenario was estimated at 2,000 mg/kg bw per day for mean consumption while at the high-level consumption was estimated at 2,600 mg/kg bw per day. Exposure estimates are the same in the refined scenario using the maximum use level reported by industry as this maximum equals the MPL. In the refined estimated exposure assessment scenario using the mean of the reported use levels from industry, exposure estimates for LBG (E 410) were of 869 mg/kg bw per day at the mean and 1,130 mg/kg bw per day at the high

level of consumption. Based on the assumption that carers of children with allergy or any other medical condition would be brand-loyal to an infant formula for special medical purposes (FC 13.1.5.1) that suits their medical condition, the Panel considers that the exposure assessment scenarios would in general result in reliable estimates of exposure.

For infants above 16 weeks of age and toddlers consuming special infant formulae (FCs 13.1.5.1 and 13.1.5.2), dietary exposure to LBG (E 410) was estimated in a FSMP consumers only scenario using the maximum reported use levels of 10,000 mg/kg for these FSMP. Mean exposure was estimated maximum at 1,107 mg/kg bw per day for infants above 16 weeks of age and 653 mg/kg bw per day for toddlers; high-level exposure (p95) was estimated maximum at 1,653 mg/kg bw per day for infants above 16 weeks of age and 1,225 mg/kg bw per day for toddlers.

The Panel considered that only very limited information with respect to the safety of the administration of E 410 could be derived from the available clinical studies because of methodological limitations. The post-marketing data provided by the IBO did not show serious adverse events among over 50 million units sold except for one single case of allergic reaction.

No new data on absorption, distribution, metabolism and excretion and no new studies on acute, subchronic, genotoxic, developmental and reproductive toxicity were provided.

A new study in neonatal piglets which were given LBG (E 410) was provided by one IBO. Changes considered related to the treatment with LBG included increased weight of segments of large and small intestines. As no related clinical signs, macroscopic and microscopic changes were observed, these changes are considered of no toxicological relevance. The changes in red blood cell parameters might be related to the observed decrease in zinc levels. Decreases in blood zinc concentration were considered of toxicological importance since zinc deficiency is known to correlate with a number of biological effects. The increase in blood glucose is considered of toxicological importance although the toxicological relevance with respect to long term adverse apical effects is less clear for the infant age and for the short life span of exposure towards LBG than in adults where micro- macrovascular changes in the retina, the kidney and blood vessels leads to blindness, renal insufficiency, and cardiovascular diseases.

Aiming at deriving a reference point, the Panel performed Benchmark dose modelling using the data from the study on neonatal piglets, i.e. the dose dependent changes in zinc and glucose levels in blood. The resulting BMDL of 1,400 mg/kg bw per day was considered as the reference point. It could also be considered to do the dose response modelling on the basis of the concentrations in formula as given in the piglet study report. This might better reflect the interaction of LBG with zinc bioavailability. Following the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010, the former ANS Panel concluded that there is no need for a numerical ADI for LBG (E 410). The FAF Panel considered that the margin of exposure (MoE) approach would be the most appropriate mean for assessing the safety of LBG (E 410) when used as a food additive in the infant and young children population. The Panel concluded based on the mechanism of the interaction between LBG and zinc that, using the reference point of 1,400 mg/kg bw per day, a MoE above 1 would not raise a safety concern.

For infants below 16 weeks of age consuming only FSMPs (FC 13.1.5.1), the MoE would be below 1 for the regulatory maximum level exposure scenario as well as for the maximum use level reported by industry; using mean use levels reported by the IBO, the MoEs would be 1.6 for mean and 1.2 for high-level consumption (95th percentile), respectively.

For infants above 16 weeks of age up to 1-year consumers only of FSMPs with a reference point of 1,400 mg/kg bw per day, the MoEs would be above 1 for the mean consumption and below 1 for the high-level consumption.

For toddlers (young children) consumers only of FSMPs, the exposure estimates calculated in the current opinion were used. The same reference point as for infants was used as the same effects related to reduced zinc absorption are expected and it was assumed that toddlers would have no higher sensitivity for decreased zinc levels. For this scenario, the MoEs would be above 1 for both the mean and high-level consumption.

Had the dose-response modelling been based on concentrations in formula the resulting conclusions would have been the same.

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## 1. Introduction

The present opinion deals with:

- The risk assessment of locust bean gum (LBG; E 410) in food for infants below 16 weeks of age in the food category (FC) 13.1.5.1 (Dietary foods for infants for special medical purposes and special formulae for infants).
- The follow-up on issues that have been expressed in the conclusions and recommendations of the Scientific Opinion on the re-evaluation of LBG (E 410) as a food additive including the risk assessment of LBG (E 410) in FC 13.1.5.2 (dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC) (EFSA ANS Panel, 2017).

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

#### 1.1.1. Background

The composition of food intended for infants and young children, as defined by Regulation (EU) No 609/2013<sup>1</sup>, is regulated at EU level and such rules include requirements concerning the use of substances as food additives.

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

In accordance with Regulation (EU) No 257/2010<sup>2</sup>, EFSA is currently re-evaluating the safety of food additives already permitted in the Union before 20 January 2009 and issuing scientific opinions on their safety when used in food as per Annexes II and III to Regulation (EC) No 1333/2008. However, the risk assessment approach followed until now has not covered the use of food additives in food for infants below 12 weeks of age. Consequently, EFSA published several scientific opinions on the re-evaluation of the safety of food additives permitted in food category 13.1 but not addressing their use in food for infants below 12 weeks of age.

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

On 31 May 2017, EFSA published a guidance document (EFSA Scientific Committee, 2017) on the risk assessment of substances present in food intended for infants below 16 weeks of age, thus enabling EFSA to assess the safety of food additives used in food for infants below 12 weeks of age.<sup>3</sup> Now EFSA is expected to launch dedicated calls for data to be able to perform such risk assessments.

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

In accordance with the current EC approach for the follow-up of EFSA's scientific opinions on the re-evaluation of the safety of permitted food additives for which some concerns have been identified, a specific call for data would be published by the EC on DG SANTE's website<sup>4</sup> on food additives and additional (missing) information would then be provided by interested business operators (IBOs) to the EC.

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

<sup>1</sup> Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. OJ L 181, 29.6.2013, pp. 35–56.

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

<sup>3</sup> See Section 1.1.3.

<sup>4</sup> [https://ec.europa.eu/food/safety/food\\_improvement\\_agents/additives/re-evaluation\\_en](https://ec.europa.eu/food/safety/food_improvement_agents/additives/re-evaluation_en)

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

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

The future evaluations of EFSA should systematically address the safety of use of food additives for all age groups, including the infants below 12 or 16 weeks of age.<sup>3</sup>

### 1.1.2. Terms of Reference

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002<sup>5</sup>, and as part of EFSA's work in completing its risk assessments concerning the use of food additives in food for infants below 12 weeks of age,<sup>3</sup> covered by the re-evaluation programme and its terms of reference, the European Commission requests the European Food Safety Authority to address all the data gaps specified in the recommendations made in this scientific opinion on the re-evaluation of the safety of food additives permitted in food category 13.1 (food for infants and young children) of annex II to Regulation (EC) No 1333/2008.

### 1.1.3. Interpretation of Terms of Reference

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

This re-evaluation refers exclusively to the authorised uses of LBG (E 410) as a food additive in food. It does not include a safety assessment of other uses of locust bean gum, such as for *ad libitum* addition of locust bean gum as a thickening agent to mother's milk or to infant formulae.

## 1.2. Previous evaluations of locust bean gum (E 410) for use in foods for infants

LBG (E 410) is authorised as a food additive in the EU according to Annexes II and III of Regulation (EC) No 1333/2008 on food additives and specific purity criteria on LBG (E 410) have been defined in Commission Regulation (EU) No 231/2012.

The food additive LBG (E 410) was not formally evaluated by the Scientific Committee on Food (SCF). However, it was considered acceptable for use in weaning food (SCF, 1991), and in infant formulae, for special medical purposes (SCF, 1994), but not for infant food in general (SCF, 1994, 1999). In 2003, the SCF re-evaluated LBG (E 410) in the revision of the essential requirements of infant formulae and follow-on formulae intended for the feeding of infants and young children (SCF, 2003). The Committee recommended maintaining the maximum level of the use of LBG in follow-on formulae of 1 g/L, while accepted that there is a case of need for use of LBG in dietary foods for special medical purposes for therapeutic use in a small number of infants with gastro-oesophageal reflux (GOR) disease under medical supervision, and the Committee considered its use in these products up to a maximum level of 10 g/L as acceptable. The Committee further recommended maintaining the concept that if more than one of the three substances LBG, guar gum or carrageenan are added to a follow-on formula, the maximum level established for each of those substances is lowered with that relative part as is present of the other substances together in that foodstuff.

LBG is also known as carob bean gum and it is by that name that JECFA refers to the additive E 410. For simplicity, only the name locust bean gum (abbreviated as LBG) is used in this Opinion for

<sup>5</sup> Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, pp. 1–24.

E 410. LBG was evaluated by JECFA in 1975, 1980 and 1981 (JECFA, 1975a,b, 1980a,b, 1981a,b). Based on the lack of adverse effects in the available toxicity studies, an ADI 'not specified' was allocated.

In 2004, the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (EFSA AFC Panel, 2004) prepared a scientific opinion on a request from the European Commission related to the use of certain food additives derived from seaweed or non-seaweed origin, including LBG (E 410) in jelly minicups. The use of these additives in jelly minicups is not authorised in the EU. Furthermore, LBG (E 410) belongs to those food additives of Group I for which the EU Regulation in general indicates that they may not be used to produce dehydrated foods intended to rehydrate on ingestion.

In 2008, JECFA updated the specifications of LBG (JECFA, 2008a,b). Purity criteria on LBG (E 410) have been defined in Commission Regulation (EU) No 231/2012 and by JECFA (JECFA, 2008a,b). As indicated by the JECFA specifications, LBG (E 410) is also produced in a purified form as clarified LBG.

In 2016, JECFA discussed the safety of use of LBG in infant formula and concluded that the available studies are not sufficient for the evaluation of LBG for use in infant formula at the proposed use level (10,000 mg/L). The Committee requested toxicological data from studies in neonatal animals, adequate to evaluate the safety for use in infant formula, to complete the evaluation (JECFA, 2016a, 2017a). According to the JECFA evaluation (JECFA, 2016a), a limit of 0.5 mg/kg for lead was introduced for LBG and LBG (clarified) for use in infant formula.

### 1.3. Summary of the previous EFSA re-evaluation of LBG (E 410) for uses in food for all population groups except for infants below 12 weeks of age<sup>6</sup>

Under the frame of Regulation (EU) No 257/2010, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) has re-evaluated the safety of LBG (E 410) when used as a food additive (EFSA ANS Panel, 2017).

In its scientific opinion, the ANS Panel reviewed available technical, biological and toxicological data on LBG (E 410) when used as a food additive. LBG was considered as not acutely toxic based on the results of acute oral toxicity studies. No adverse effects were reported in 90-day toxicity and carcinogenicity studies in rodents at the highest doses (20,000 mg LBG/kg body weight (bw) per day in mice, and 4,500 or 9,000 mg LBG/kg bw per day in rats in 90-day toxicity studies; up to 7,500 mg LBG/kg bw per day in mice and 2,500 mg LBG/kg bw per day in rats in carcinogenicity studies) tested and there was no concern with respect to the genotoxicity and to reproductive and developmental toxicity of LBG (E 410). The ANS Panel concluded that there is no need for a numerical acceptable daily intake (ADI) for LBG (E 410), following 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 there is no safety concern for the general population at the refined exposure assessment for the reported uses of LBG (E 410) as a food additive.

The ANS Panel noted reports suggesting a putative effect of LBG to decrease the bioavailability of certain nutrients (calcium, iron and zinc); however, a human study did not confirm this effect.

For the specific group of infants of more than 12 weeks of age, the ANS Panel considered a case of sensitivity and reports on undesirable gastrointestinal (GI) effects, such as diarrhoea, frequent loose stools and flatulence, associated with the use of LBG in products for reduction in GOR. Furthermore, the ANS Panel noted that no specific clinical data addressing the safety of use of LBG (E 410) in 'dietary foods for infants for special medical purposes and special formulae for infants' (food category 13.1.5.1) and in 'dietary foods for baby and young children for special medical purposes as defined in Directive 1999/21/EC' (food category 13.1.5.2) considering the defined maximum use levels were available to the ANS Panel.

The ANS Panel also noted that infants and young children consuming these foods may be exposed to a greater extent to LBG (E 410) than their healthy counterparts because the permitted levels of LBG (E 410) in products for special medical purposes to reduce GOR are 10-fold higher than in follow-on formulae for healthy individuals. The ANS Panel further noted that, given their medical condition, infants and young children consuming foods belonging to these food categories may show a higher susceptibility to the GI effects of LBG than their healthy counterparts. Thus, monitoring of any adverse

<sup>6</sup> According to the EFSA Scientific Committee Guidance (EFSA Scientific Committee, 2017) this opinion will include infants up to 16 weeks of age, see Section 1.1.3.

effects including those in the GI system in infants and young children consuming these foods under medical supervision could be helpful to reduce this uncertainty.

Overall, the ANS Panel concluded that the available data do not allow an adequate assessment of the safety of LBG (E 410) in infants and young children consuming foods for special medical purposes. The ANS Panel further considered that the conclusions reached on the re-evaluation of the food additive were not applicable to the use of LBG (E 410) in food for infants under the age of 12 weeks.<sup>7</sup> The ANS Panel considered that these uses would require a specific risk assessment.

In addition, the following recommendations relevant for this evaluation were issued by the ANS Panel:

- The maximum limits for the impurities of toxic elements (lead, mercury and arsenic) in the EU specification for LBG (E 410) should be revised in order to ensure that LBG (E 410) as a food additive will not be a significant source of exposure to those toxic elements in food in particular for infants and children.
- Separate specifications should be given in the EU regulation for LBG and clarified LBG differing significantly in the protein content.
- The LBG protein content should be reduced as much as possible due to some case reports of hypersensitivity reactions associated with LBG (E 410).
- The microbiological specifications in the EU Regulation for polysaccharidic thickening agents, such as gums, should be harmonised and criteria for the absence of *Salmonella* spp. and *E. coli*, for total aerobic microbial count (TAMC) and for total combined yeast and mould count (TYMC) should be included into the EU specifications of LBG (E 410).
- In view of the synergistic effects on viscosity and gelling with mixtures of LBG (E 410) and carrageenan (E 407), carrageenan (E 407) should be included in the footnote for food category 13.1.4 regulating the combined use of the gums.
- Concerning the direct use of LBG (E 410) by the consumer in the form of a powder to thicken food, the ANS Panel recommended labelling, including instructions for adequate preparation of the thickened food with sufficient liquid to avoid the risk of possible oesophageal obstruction due to insufficient hydration of the LBG.
- Additional data should be generated to assess the potential health effects of LBG (E 410) when used in 'dietary foods for infants for special medical purposes and special formulae for infants' (Food category 13.1.5.1) and in 'dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC' (Food category 13.1.5.2).

## 2. Data and methodologies

### 2.1. Data

EFSA launched a public call for data<sup>8</sup> to collect relevant information from the IBOs. Additional clarification or supplemental information were provided to EFSA by the IBOs that had replied to the call for data and during a hearing held at the 20th meeting of the FAF Panel Working Group (WG) on food additives permitted in foods for infants below 16 weeks of age.<sup>9</sup>

The Panel based its assessment on information submitted to EFSA following the public call for data and the conclusions and recommendations from previous evaluations.

To verify the use of LBG (E 410) in food products the Mintel's Global New Products Database (GNPD) was used.

### 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, 2009) and following the relevant existing guidance documents from the EFSA Scientific Committee and in particular the EFSA

<sup>7</sup> According to the EFSA Scientific Committee Guidance (EFSA Scientific Committee, 2017) this opinion will include infants up to 16 weeks of age.

<sup>8</sup> Call for technical and toxicological data on locust bean gum (E 410) as a food additive for uses in foods for all population groups including infants below 16 weeks of age. Published: 18 July 2018. Available from: <https://www.efsa.europa.eu/en/consultations/call/call-technical-and-toxicological-data-locust-bean-gum-e-410-uses>

<sup>9</sup> <https://www.efsa.europa.eu/sites/default/files/wgs/food-ingredients-and-packaging/wg-foodadditivesinfants-m.pdf>

Guidance of the Scientific Committee on the risk assessment of substances present in food intended for infants below 16 weeks of age (EFSA Scientific Committee, 2017).

In order to conclude on the safety of LBG (E 410) for all population groups and to address the data gaps identified during the re-evaluation, the FAF Panel assessed the information provided:

- For the risk assessment of LBG (E 410) in food for infants below 16 weeks of age in the food category (FC) 13.1.5.1 (Dietary foods for infants for special medical purposes and special formulae for infants).
- For the follow-up on issues that have been expressed in the conclusions and recommendations of the Scientific Opinion on the re-evaluation of LBG (E 410) as a food additive including the risk assessment of LBG (E 410) in FC 13.1.5.2 (dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC) (EFSA ANS Panel, 2017).

When in animal studies, the test substance was administered in the feed or in 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, 2012) are applied. In the case of other animal species, the default values 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 human studies in adults (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, 2012).

The study on neonatal piglets was assessed for risk of bias (RoB) by two reviewers (members of the FAF Panel WG on food additives permitted in foods for infants below 16 weeks of age) applying an assessment tool modified from the OHAT RoB tool (NTP, 2015, 2019). The elements considered for the appraisal are described in the Appendix B to this opinion, as well as the decision rule for assigning the studies to Tiers of reliability. The human studies were not subject to a formal risk of bias assessment since all available studies were uncontrolled and unblinded. The Panel considered that such study design would qualify the studies as having a high risk of bias.

The benchmark dose (BMD) approach (see Section 3.5.4 and Appendices C and D) was applied to the data from the neonatal piglet study for deriving the reference point (RP) in line with the EFSA Guidance (EFSA SC, 2017<sup>10</sup>).

Dietary exposure to LBG (E 410) from its use as a food additive in foods for infants below 16 weeks of age was estimated combining the mean and high-level consumption values reported for the period of 14–27 days of life which corresponds to 200 and 260 mL/kg bw per day (EFSA Scientific Committee, 2017), respectively, with the maximum levels according to Annex II and reported use levels submitted to EFSA following a call for data. Different scenarios were used to calculate exposure (see Section 3.3.1). Uncertainties on the exposure assessment were identified and discussed (see Section 3.5.3).

As LBG (E 410) is also authorised in the food category 13.1.5.2, an exposure assessment considering FC 13.1.5.1 and FC 13.1.5.2 was performed to estimate the exposure of infants (above 16 weeks) and toddlers who may eat and drink these foods for special medical purposes (FSMP). The consumption of these foods is not reported in the EFSA Comprehensive database. To consider potential exposure to LBG (E 410) via these foods, the Panel assumes that the amount of FSMP consumed by infants and toddlers resembles that of comparable foods in infants and toddlers from the general population. Thus, the consumption of FSMP categorised as FC 13.1.5 was assumed equal to that of formulae and food products categorised as FCs 13.1.1, 13.1.2, 13.1.3 and 13.1.4.

<sup>10</sup> The Panel is aware of the recently published 'Guidance on the use of the benchmark dose approach in risk assessment' (EFSA Scientific Committee, 2022), however, this Guidance was published when section 3.5.4 and Appendices C and D of the scientific opinion were already agreed and finalised and an update was considered not needed.

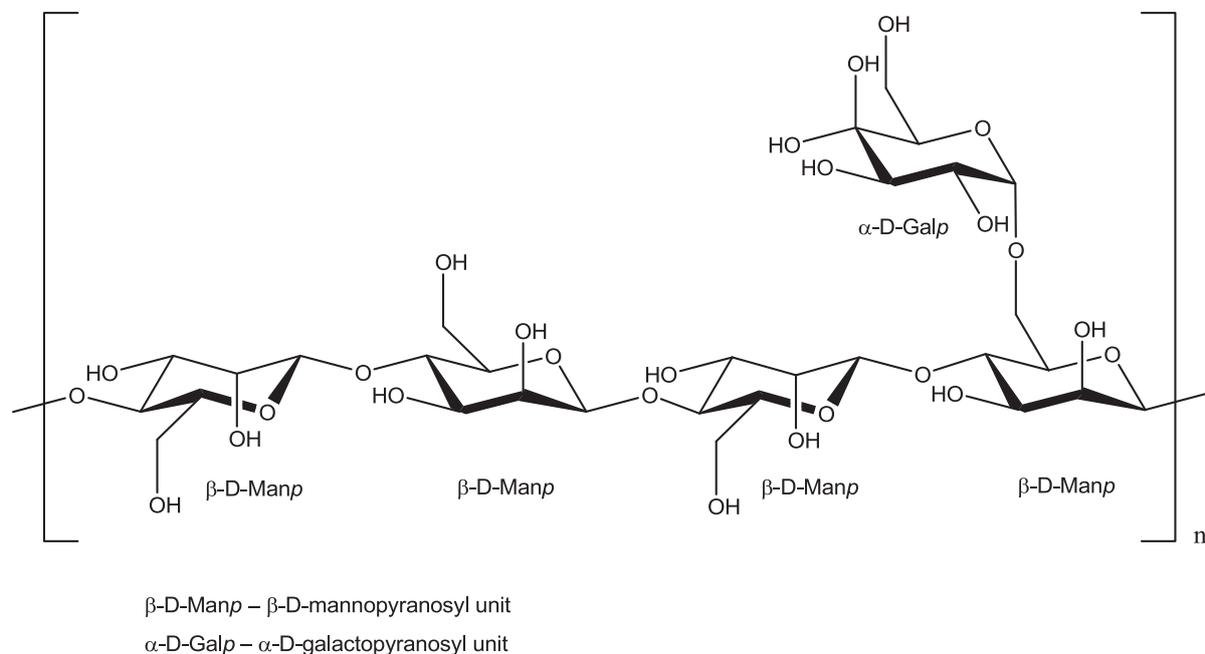
### 3. Assessment

#### 3.1. Identity and specifications of E 410

According to Commission Regulation (EU) No 231/2012<sup>11</sup>, the food additive E 410 is named as locust bean gum (synonyms: carob bean gum and algaroba gum) and is the ground endosperm of the seeds of the strains of carob tree, *Ceratonia siliqua* (L.) Taub. (Family Leguminosae). The substance has the CAS Registry number 9000-40-2 and the EINECS number 232-541-5. The molecular weight of LBG varies over a wide range and is reported to be 50,000–3,000,000 g/mol (Commission Regulation (EU) No 231/2012). JECFA refers to the additive E 410 Locust bean gum as carob bean gum. For simplicity, only the name locust bean gum (abbreviated as LBG) is used in this Opinion for E 410.

Locust bean gum (LBG) is also produced in purified form as 'Carob Bean Gum (Clarified)' INS No 410 (JECFA, 2016b). According to the JECFA specifications, clarified LBG does not contain cell wall materials and the content of protein is less than 1% (JECFA, 2016b). In comparison, LBG according to JECFA and EU specifications may contain up to 7% of protein. An IBO confirmed that according to the results for purity testing refined LBG complies with the requirements for clarified LBG from JECFA specifications (Documentation provided to EFSA, n. 4).

The structural formula of polysaccharide units of LBG is presented in Figure 1 (as presented in EFSA ANS Panel, 2017).



**Figure 1:** Structural formula of polysaccharide units of LBG (EFSA ANS Panel, 2017)

The specifications for LBG (E 410) as defined in the Commission Regulation (EU) No 231/2012 and as proposed by JECFA (2016b) are listed in Table 1.

<sup>11</sup> 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 Text with EEA relevance. OJ L 83, 22.3.2012, pp. 1–295.

**Table 1:** Specifications for locust bean gum (E 410) according to Commission Regulation (EU) No 231/2012 and proposed by JECFA (2016b)

	<b>Commission Regulation (EU) No 231/2012</b>	<b>JECFA (2016b/2017b) (carob bean gum)<sup>12</sup></b>	<b>JECFA (2016b) (clarified carob bean gum)</b>
Definition	Locust bean gum is the ground endosperm of the seeds of the strains of carob tree, <i>Ceratonia siliqua</i> (L.) Taub. (Family Leguminosae). Consists mainly of a high molecular weight hydrocolloidal polysaccharide, composed of galactopyranose and mannopyranose units combined through glycosidic linkages, which may be described chemically as galactomannan.	Carob bean gum, also known as locust bean gum, is a galactomannan polysaccharide obtained from the seeds of <i>Ceratonia siliqua</i> (L.) Taub. (Fam. Leguminosae). The ground endosperm of the seeds consists mainly of high molecular weight (approximately 50,000–3,000,000) polysaccharides composed of galactomannans with a mannose:galactose ratio of about 4:1. The seeds are dehusked by treating the seeds with dilute sulfuric acid or with thermal mechanical treatments, elimination of the germ followed by milling and screening of the endosperm to obtain native carob bean gum. The gum may be washed with ethanol or isopropanol to control the microbiological load (washed carob bean gum).	Carob bean gum, also known as locust bean gum, is a galactomannan polysaccharide obtained from the seeds of <i>Ceratonia siliqua</i> (L.) Taub. (Fam. Leguminosae). The ground endosperm consists mainly of high molecular weight (approximately 50,000–3,000,000) polysaccharides composed of galactomannans with a mannose:galactose ratio of about 4:1. The seeds are dehusked by treating the seeds with dilute sulfuric acid or with thermal mechanical treatments, elimination of the germ, followed by milling and screening of the endosperm to obtain native carob bean gum. The gum is clarified by dispersing in hot water, filtration and precipitation with ethanol or isopropanol, filtering, drying and milling. The clarified carob bean gum does not contain cell wall materials. Clarified carob bean gum in the market is normally standardised with sugars for viscosity and reactivity.
Molecular weight	50,000–3,000,000	–	–
Assay	Galactomannan content not less than 75%	–	–
Description	White to yellowish-white, nearly odourless powder	White to yellowish white, nearly odourless powder	White to yellowish white, nearly odourless powder
Functional uses	–	Stabiliser, thickener, emulsifier, gelling agent	Stabiliser, thickener, emulsifier, gelling agent
<b>Identification</b>			
Test for galactose	Passes test	–	–
Test for mannose	Passes test	–	–
Solubility	Soluble in hot water, insoluble in ethanol	Insoluble in ethanol	Insoluble in ethanol
Gel formation		Add small amounts of sodium borate TS to an aqueous dispersion of the sample; a gel is formed.	Add small amounts of sodium borate TS to an aqueous dispersion of the sample; a gel is formed.
Viscosity	–	Transfer 2 g of the sample into a 400-mL beaker and moisten thoroughly with	Transfer 2 g of the sample into a 400-mL beaker and moisten thoroughly with about

<sup>12</sup> JECFA refers to the additive E 410 Locust bean gum as carob bean gum. For simplicity, only the name locust bean gum is used in this Opinion for E 410. The Panel noted that the IBOs referred in their submission to locust bean gum as to unclarified locust bean gum. Therefore, for the sake of readability the term of the unclarified locust bean gum refers in this opinion to locust bean gum or carob ben gum.

	Commission Regulation (EU) No 231/2012	JECFA (2016b2017b) (carob bean gum) <sup>12</sup>	JECFA (2016b) (clarified carob bean gum)
		about 4 mL of isopropanol. Add 200 mL of water with vigorous stirring until the gum is completely and uniformly dispersed. An opalescent, slightly viscous solution is formed. Transfer 100 mL of this solution into another 400-mL beaker. Heat the mixture in a boiling water bath for about 10 min and cool to room temperature. There is an appreciable increase in viscosity (differentiating carob bean gums from guar gums).	4 mL of isopropanol. Add 200 mL of water with vigorous stirring until the gum is completely and uniformly dispersed. An opalescent, slightly viscous solution is formed. Transfer 100 mL of this solution into another 400-mL beaker. Heat the mixture in a boiling water bath for about 10 min and cool to room temperature. There is an appreciable increase in viscosity (differentiating carob bean gums from guar gums).
Gum constituents	–	(a)	(a)
Microscopic examination	Place some ground sample in an aqueous solution containing 0.5% iodine and 1% potassium iodide on a glass slide and examine under microscope. Locust bean gum contains long stretched tubiform cells, separated or slightly interspaced. Their brown contents are much less regularly formed than in guar gum. Guar gum shows close groups of round to pear shaped cells. Their contents are yellow to brown.	Disperse a sample of the gum in an aqueous solution containing 0.5% iodine and 1% potassium iodide on a glass slide and examine under a microscope. Carob bean gum contains long stretched tubiform cells, separated or slightly interspaced. Their brown contents are much less regularly formed than in Guar gum.	–
<b>Purity</b>			
Loss on drying	Not more than 15% (105°C, 5 h)	Not more than 14% (105°, 5 h)	Not more than 14% (105°, 5 h)
Ash	Not more than 1.2% determined at 800°C	Not more than 1.2% (800°, 3–4 h)	Not more than 1.2% (800°, 3–4 h)
Acid-insoluble matter	Not more than 4%	Not more than 4.0%	Not more than 3.5%
Protein (N × 6.25)	Not more than 7%	Not more than 7.0% (Kjeldahl Method) <sup>(a)</sup>	Not more than 1.0% (Kjeldahl Method) <sup>(a)</sup> .
Starch	Not detectable by the following method: to a 1 in 10 solution of the sample add a few drops of iodine solution. No blue colour is produced.	To a 1 in 10 dispersion of the sample add a few drops of iodine TS; no blue colour is produced.	To a 1 in 10 solution of the sample add a few drops of iodine TS; no blue colour is produced.
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg. Not more than 0.5 mg/kg for use in infant formula and in formulae for special medical purposes intended for infants. <sup>(a)</sup>	Not more than 2 mg/kg. Not more than 0.5 mg/kg for use in infant formula and in formulae for special medical purposes intended for infants. <sup>(a)</sup>
Arsenic	Not more than 3 mg/kg	Not more than 3 mg/kg. <sup>(a)</sup>	Not more than 3 mg/kg. <sup>(a)</sup>
Mercury	Not more than 1 mg/kg	–	–
Cadmium	Not more than 1 mg/kg	–	–

	Commission Regulation (EU) No 231/2012	JECFA (2016b2017b) (carob bean gum) <sup>12</sup>	JECFA (2016b) (clarified carob bean gum)
Ethanol and propan-2-ol	Not more than 1%, single or in combination	Not more than 1% of ethanol or isopropanol, singly or in combination. <sup>(a)</sup>	Not more than 1% of ethanol or isopropanol, singly or in combination. <sup>(a)</sup>
Microbiological criteria	–	Total (aerobic) plate count: Not more than 5,000 CFU/g. <i>E. coli</i> : Negative in 1 g. <i>Salmonella</i> : Negative in 25 g. Yeasts and moulds: Not more than 500 CFU/g. <sup>(a)</sup>	Total (aerobic) plate count: Not more than 5,000 CFU/g. <i>E. coli</i> : Negative in 1 g. <i>Salmonella</i> : Negative in 25 g. Yeasts and moulds: Not more than 500 CFU/g. <sup>(a)</sup>

CFU: colony forming units.

(a): Further information on the test methods to be used is provided in the JECFA specifications directly and/or by reference to 'Volume 4 (under "General Methods")' [JECFA, 2004, 2016b]. These method details are omitted here for reasons of brevity and clarity.

The revisions of the existing EU specifications proposed by the Panel are provided under Section 3.4.

### 3.2. Technical data submitted

In order to support the revision of the existing specifications, the Panel has assessed the data provided by the IBOs in response to the EFSA call for data.<sup>8</sup>

The Panel noted that in the JECFA specifications a distinction is made between LBG and clarified LBG (Table 1) whereas no such distinction is made in the EU definition and specifications of LBG (E 410). Furthermore, the EFSA ANS Panel in 2017 recommended to explore the need to separate the current EU specifications of E 410 to locust bean and clarified LBG. The Panel noted that the IBOs referred in their submission to 'locust bean gum' as described in the JECFA specifications as to 'unclarified' LBG in line with the call for data.<sup>8</sup> Therefore, for the sake of readability the technical data submitted by IBO were ascribed in this opinion either to unclarified or to clarified LBG.

In the response to the call for data, two IBOs (Documentation provided to EFSA n. 1, 2, 3) submitted analytical data on the levels of toxic elements, residual protein and microbiological data in unclarified LBG (E 410) and one IBO provided data for clarified LBG (E 410) (Documentation provided to EFSA n. 5).

#### 3.2.1. Toxic elements

The following was requested in the EFSA call for data:

- Analytical data on current levels of lead, mercury, cadmium and arsenic in commercial samples of the food additive E 410.
- The lowest technologically achievable level for lead, mercury, cadmium, and arsenic in order to adequately define their maximum limits in the specifications.

#### Unclarified LBG

One IBO representing producers of unclarified LBG provided analytical data on levels of lead (Pb), mercury (Hg), cadmium (Cd) and arsenic (As) in commercial samples of unclarified LBG (E 410) (Documentation provided to EFSA n. 1 and 2). Summary data were reported for 'more than 35 samples' of E 410 coming from five member companies covering the period 2018–2020. A large number of analytical methods were reported, mainly based on inductively coupled plasma mass spectrometry (ICP-MS) but some using atomic absorption spectroscopy (AAS) and with a variety of sample preparation methods used. There was no breakdown of results for individual batches, individual companies, or the individual methods. The levels of Pb, Cd and As in all batches were reported as below the respective limits of quantification (LOQs) which were in the range of 0.01–0.1 mg/kg for all three elements (Documentation provided to EFSA n. 2). Similarly, the content of Hg in all batches was reported as below the LOQs which were in the range of 0.005–0.1 mg/kg.

The same IBO reported that the lowest technologically achievable levels depend on the raw materials used, since during the manufacturing of unclarified LBG there are no specific processes which would reduce heavy metal levels. The lowest technologically achievable levels of Pb, Hg, Cd and

As proposed by the IBO for unclarified LBG used in foods for all population groups including infants are shown in Table 2 (Documentation provided to EFSA n. 1).

**Table 2:** Lowest technologically achievable levels for the toxic elements lead, mercury, cadmium and arsenic in commercial unclarified E 410 for all population groups including infants below 16 weeks of age, as proposed by an IBO (Documentation provided to EFSA n. 1)

Lead	Mercury	Cadmium	Arsenic
0.5 mg/kg	0.5 mg/kg	0.25 mg/kg	1.5 mg/kg

IBO: interested business operator.

The Panel noted that the lowest technologically achievable levels proposed by the IBO for Pb, Hg, Cd and As are much higher than most of the reported levels measured in commercial batches of unclarified E 410.

Another IBO provided analytical data on six batches of unclarified LBG (E 410) covering the production period of 2018 and 2019 (Documentation provided to EFSA n. 3). The elements were determined using an ICP-MS method. The content of Cd, Hg and As was reported as below the LOQ which was 0.005 mg/kg for each of the three elements. Pb was measurable in all six batches at 0.011, 0.026, 0.038, 0.038, 0.040 and 0.101 mg/kg, and the LOQ was 0.005 mg/kg.

This IBO did not provide the lowest technologically achievable levels for toxic elements in unclarified LBG E 410 but proposed the JECFA specifications for carob bean gum, which includes only the specifications for Pb (2 mg/kg for all population and a maximum limit of 0.5 mg/kg for Pb specification for infant formulae) and As (3 mg/kg). The Panel noted that the maximum limit of Pb at 2 mg/kg and As at 3 mg/kg is also included in the current EU specifications for E410 (Documentation provided to EFSA n. 3) and are substantially higher than the reported levels measured in commercial batches of unclarified E 410.

### Clarified LBG

One IBO provided data on the contents of Pb, Hg, Cd and As in five batches of clarified LBG (E 410) (Documentation provided to EFSA n. 5). The levels of the elements were determined by using an in-house method based on ICP-MS. For all 5 batches analysed the levels of Hg, Cd and As were reported as below the LOQs of 0.046, 0.034 and 0.112 mg/kg, respectively. The level of Pb was measurable in all 5 batches at 0.021, 0.031, 0.044, 0.37 and 0.77 mg/kg, and the LOQ was 0.02 mg/kg.

The IBO stated that in the production process of clarified LBG, the unclarified gum is used as a raw material undergoes purification. According to the IBO, the levels of toxic elements in the food additive E 410 are mainly dependent on their concentrations in the raw materials, processing aids, the conditions used in the manufacturing process as well as geographical and seasonal variations dependent on where the carob trees are grown. The IBO stated that some of the manufacturing steps, both in the processing of the raw material (i.e. unclarified LBG) and the process of clarifying the LBG e.g., aqueous extraction at elevated temperatures and alcohol precipitation 'may remove some of the toxic metal elements'. The lowest technologically achievable levels for toxic elements in clarified LBG (E 410) proposed by the IBO are reported in Table 3 (Documentation provided to EFSA n. 5).

**Table 3:** Lowest technologically achievable levels for the toxic elements lead, mercury, cadmium and arsenic in commercial clarified E 410, as proposed by an IBO (Documentation provided to EFSA n. 5)

Lead	Mercury	Cadmium	Arsenic
1 mg/kg	0.1 mg/kg	0.1 mg/kg	0.3 mg/kg

IBO: interested business operator.

The Panel considered that the limit values proposed by the IBO reported for clarified E 410 seem reasonable compared with the analytical data provided for production samples, when taking into account the need to provide some 'headroom' to account for representativeness, homogeneity and analytical measurement uncertainty.

### 3.2.2. Residual proteins

The following was requested in the EFSA call for data:

- Current levels of residual proteins in clarified and unclarified preparations;
- The possibility to use clarified locust bean gum to cover all technological needs of the food additive E 410;
- The lowest technologically achievable level for residual proteins in clarified and unclarified preparations in order to adequately define their maximum limits in the specifications in view of case reports on hypersensitivity reactions associated with locust bean gum;
- A proposal for separate specifications for clarified and unclarified locust bean gum is requested.

#### Unclarified LBG

One IBO, the association representing producers of unclarified locust bean gum, reported the residual protein content in 30 commercial batches of unclarified LBG measured by the Kjeldahl method (Documentation provided to EFSA n. 1). The range of values was given as from 4.5% to 7.0% w/w of protein, but no individual results were provided.

Another IBO reported levels of residual protein determined in six batches of unclarified LBG measured using an in-house method (a modified Kjeldahl method). The results ranged between 6.28 and 7.0% w/w (Documentation provided to EFSA n. 3).

According to one IBO, the lowest technologically achievable level for residual protein depends on the levels that are present in the raw material used to manufacture the food additive. They stated that during the manufacturing of unclarified LBG there are no specific steps which would reduce the protein level. Therefore, they supported the existing specification for E 410 which limits the residual protein to 7% (Table 1) (Documentation provided to EFSA n. 1).

Similarly, the other IBO reported that the endosperm fraction used for production of unclarified LBG can have protein content up to 7% and it is not possible to remove it further with the current manufacturing process for unclarified E 410. According to this IBO, further reduction of the protein content would require an additional manufacturing step and this would result in clarified LBG (Documentation provided to EFSA n. 3).

#### Clarified LBG

One IBO stated that the protein content in clarified LBG is usually less than 1%. In five reported batches of clarified LBG analysed by the Kjeldahl method, the protein content was ranging between 0.31 and 0.50% w/w (Documentation provided to EFSA n.5).

This IBO described that LBG originates from the seeds of the carob tree and so proteins and other plant constituents may be carried over to the final product. As reported by the IBO, lower protein content may be obtained in clarified E 410 as the majority of protein is removed during the production process by way of an aqueous extraction at elevated temperatures and an alcohol precipitation. Consequently, the lowest technologically achievable level for residual protein will be different in clarified and unclarified preparations. For clarified LBG, the IBO proposed a maximum value of 1 % for protein residues (Documentation provided to EFSA n. 5).

The IBO also noted that JECFA has separate specifications for unclarified and clarified LBG (see Table 1) and they supported having separate specifications (Documentation provided to EFSA n. 5). To further support this distinction and to highlight the differences in composition, the IBO provided information on the purity of batches of feedstock (unclarified LBG) and batches of clarified E 410 produced (see Table 4).

**Table 4:** Comparison between the average purity values of an unclarified LBG used as a raw material and clarified LBG as provided by the IBO (Documentation provided to EFSA n. 5)

	Average values for unclarified LBG used as a raw material (based on 8 batches)	Average values for clarified LBG (based on 12 batches of IBO product)
Galactomannan (%)	81.12	93.6
Protein (%)	6.62	0.37

	Average values for unclarified LBG used as a raw material (based on 8 batches)	Average values for clarified LBG (based on 12 batches of IBO product)
Acid-insoluble matter (%)	0.92	0.36
Ash (%)	1.07	0.36
Moisture/loss on drying (%)	10.27	5.32
Ethanol and 1-propanol (%)	< 1	0.14
Aerobic Plate Count (TAMC)	1227 <sup>(a)</sup>	18 <sup>(a)</sup>
Yeast & Moulds (TYMC)	67 <sup>(a)</sup>	2 <sup>(a)</sup>

TAMC: total aerobic microbial count; TYMC: total combined yeast and mould count.

(a): According to the test method description provided by the IBO CFU/g (Documentation provided to EFSA n. 5).

As reported by the same IBO (Documentation provided to EFSA n. 5 and 6), the major differences between unclarified and clarified LBG are that the gum content (galactomannan) is higher, and the protein content is lower after the clarification process. Lower levels of acid-insoluble matter, ash, moisture, TAMC and TYMC are also achieved (Table 4). The specifications for clarified LBG proposed by this IBO are summarised in Table 5.

**Table 5:** Comparison between the existing EU specifications and the specifications for clarified E 410 as proposed by an interested business operator (Documentation provided to EFSA n. 5)

	E 410 LBG according to EU 231/2012	Specifications for clarified LBG proposed by an IBO
Protein	NMT <sup>(a)</sup> 7%	1%
Acid-insoluble matter	NMT 4%	1%
Total ash	NMT 1.2 % determined at 800°C	0.6%
Loss on drying	NMT 15 % (105°C, 5 h)	14%
Residual solvents (ethanol or 2-propanol)	NMT 1 %, single or in combination	0.5 %, single or in combination
Aerobic Plate Count (TAMC)	–	300 <sup>(b)</sup>
Yeast & Moulds (TYMC)	–	100 <sup>(b)</sup>
<i>E. coli</i>	–	Negative in 1 g
<i>Salmonella</i>	–	Negative in 25 g
Lead	NMT 2 mg/kg	NMT 1 mg/kg
Arsenic	NMT 3 mg/kg	NMT 0.3 mg/kg
Mercury	NMT 1 mg/kg	NMT 0.1 mg/kg
Cadmium	NMT 1 mg/kg	NMT 0.1 mg/kg
Starch	Not detectable (by the method described.)	Not detectable (same method as EU)

TAMC: total aerobic microbial count; TYMC: total combined yeast and mould count.

(a): NMT: not more than.

(b): The Panel noted that these values most likely represent CFU/g.

### 3.2.3. Microbiological criteria

The following was requested in the EFSA call for data:

- Because of both the botanical origin and the polysaccharidic nature of locust bean gum, it can be a substrate of microbiological contamination. Data should be provided demonstrating the absence of *Salmonella* spp. and *Escherichia coli* and on the lowest TAMC and TYMC that can be reached.
- Data should be provided demonstrating the absence of *Cronobacter (Enterobacter) sakazakii*.

### Unclarified LBG

One IBO, an association representing members that produce only unclarified LBG reported the results of microbiological analyses of 'more than 50 batches' of unclarified LBG and *Salmonella* spp. was not detected (neg/25 g). In the analysis of 'more than 35 batches' of unclarified LBG *Escherichia*

*coli*, was also not detected (neg/1 g). According to the IBO, the lowest technologically achievable levels for total TAMC and TYMC in unclarified LBG are 5,000 and 500 CFU/g, respectively, as set in the JECFA specifications (Documentation provided to EFSA n.1).

Another IBO provided data on five batches of unclarified LBG. *Salmonella* spp. and *Escherichia coli* were not detected in both 25 g and 1 g samples (Documentation provided to EFSA n. 3). The levels of TAMC and TYMC determined for five batches were for TAMC 40, 80, 200, 320 and 320 CFU/g and for TYMC for three batches were reported as < 40 CFU/g and for remaining two as 150 and 460 CFU/g. The IBO did not submit proposals for the lowest technologically achievable levels for TAMC and TYMC but simply stated that the five batches meet the JECFA specifications (JECFA, 2016b, 2017b).

One IBO submitted data demonstrating the absence of *Cronobacter* (*Enterobacter*) *sakazakii* in 30 batches (spanning 6 months in 2017) of unclarified LBG; (neg/10 g) (Documentation provided to EFSA n. 3 and 4). All samples were analysed by an external accredited laboratory in 2017 using the method in force at that time (Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs,<sup>13</sup> *Cronobacter sakazakii* was analysed by ISO/TS 22964:2006 (IDF/RM 210: 2006)). Since March 2018, the Regulation changed and *Cronobacter* spp. is tested for by ISO 22964:2017.

### Clarified LBG

According to another IBO, the LBG can be a substrate of microbiological contamination and of field and storage fungal development. The IBO further stated that purification process of clarified LBG (aqueous extraction at elevated temperatures, alcohol precipitation and drying) ensures a low microbial count in the product. For five batches of clarified LBG, TAMC values were 10 CFU/g for four batches, and 40 CFU/g for one batch. TYMC values were 1 CFU/g for three batches, and for two remaining 4 and 5 CFU/g. Both *E. coli* (neg/5 g) and *Salmonella* (neg/25 g) were not detected in the samples (Documentation provided to EFSA n. 6).

The Panel noted that the values reported by the IBOs for microbiological count for both clarified and unclarified LBG (E 410) meet the criteria as set in JECFA specifications (JECFA, 2016b, 2017b).

### 3.2.4. Information on particular specifications requirements in the additive for use in infant formulae

The following was requested in the EFSA call for data:

- Information on particular specification requirements for identity and the purity of locust bean gum (E 410) (e.g. with respect to levels of protein residues; use of clarified locust bean gum; content of toxic elements, propan-2-ol/ethanol) for special formulae for infants below 16 weeks of age under special medical conditions;
- Analytical data on impurities in the final special formulae for infants below 16 weeks of age need to be provided when no legal limit has been established.

Information was submitted for unclarified LBG (E 410) but no information was provided for the clarified LBG (E 410) in response to this specific request.

One IBO provided ICP-MS data on levels of Hg and As in 77 batches of an infant FSMP containing unclarified LBG (E 410). The use level of the additive was not reported. All results for Hg and As in the FSMP product were reported as below the LOQ which was 0.01 mg/kg for each element (Documentation provided to EFSA n. 3).

The same IBO stated that, to the best of its knowledge, only unclarified LBG is used in infant formulae (Documentation submitted to EFSA n. 4). The IBO proposed to align the specifications for unclarified LBG intended for use in FSMP with the corresponding JECFA specifications (JECFA, 2016b, 2017b) (see Table 1 in Section 3.1).

The IBO provided the results of the analysis of propan-2-ol in two batches of E 410 as below the LOQ of 0.5 % w/w. According to the IBO, ethanol is not used in the manufacturing process for unclarified LBG and, therefore, data on the ethanol content were not provided. No further particular specifications requirements for E 410 specifically intended for uses in foods for infants were put forward by the IBO.

<sup>13</sup> Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs (Text with EEA relevance). OJ L 338, 22.12.2005, p. 1–26.

### 3.2.5. Stability of the substance, and reaction and fate in food

The following was requested in the EFSA call for data:

- Information on the fate and the reaction products of locust bean gum (E 410) in special formulae for infants below 16 weeks of age under special medical conditions (FC 13.1.5.1).

According to one IBO currently, there is no information available pertaining to the fate and reaction products of LBG specifically in FSMP (Documentation submitted to EFSA n. 3). The IBO stated that based on the accessible data, formation of reaction products as a result of interaction with LBG within the food matrices is not anticipated. The IBO provided a description on the physical fate of E 410 that is summarised as follows: LBG is only partially soluble in water with 50% solubilisation of an initial 0.1% w/w dispersion after 1 h stirring at room temperature (Richardson et al., 1998; Dakia et al., 2008). LBG may form viscous solutions at relatively low concentrations, which are almost unaffected by pH (within the range of 3–11), by salts, or by temperature (Alves et al., 1999; Beneke et al., 2009). LBG has been used in baked goods, such as bread, rolls, cakes and cookies indicating that it is stable and maintains functionality at high temperatures during cooking/baking process (Miś and Dżiki, 2013). Reactions of gums with skim-milk and with purified proteins were investigated using polyacrylamide gel (PAG) electrophoresis and centrifugation (Grindrod and Nickerson, 1968). LBG caused wheying-off or coprecipitation, but electrophoretic migration of proteins was unaffected. This indicates that LBG may not react with milk proteins in infant FSMP. The viscosity of LBG suspensions was not affected when prepared at a range of pH values, from 5.0 to 9.0 at ambient temperature (Kök, 2007) but heat treatment at 100°C for 30 min with the same range of pH values resulted in relatively greater degradation causing loss of viscosity, which was attributed to depolymerisation. LBG has been shown to interact synergistically with other polysaccharides such as xanthan gum and carrageenan regarding the gelling properties (Sewall, 1992; Dakia et al., 2008). The synergism could be primarily due to the numerous hydroxyl groups present in the molecule structure (Srivastava and Kapoor, 2005). This synergy increases the flexibility of the polymer and, in many cases, permits the production of gelling structures.

Furthermore, the same IBO stated that 'in connection with this 'synergy', the JECFA (2016b) Definition of carob (locust) bean gum (clarified, see Table 1) ends with the sentence: Clarified carob bean gum in the market is normally standardised with sugars for viscosity and reactivity' The three IBOs were requested to provide clarification on what is meant in that JECFA definition by 'reactivity'. The IBOs explained that the term does not relate to any chemical reaction. Rather, it relates to the dissolution properties of clarified LBG in combination with other hydrocolloids and the synergistic effects of LBG with other hydrocolloids (i.e. xanthan gum or carrageenan) where for example a gel formed by use of equal parts of LBG and carrageenan is firmer/stronger than a gel with carrageenan or LBG alone (Documentation submitted to EFSA n. 3).

### 3.2.6. Solubility

No data were provided on the solubility of E 410 in water. The IBOs stated that they encountered difficulties in measuring the solubility of E 410 in water by applying the test method as described in the OECD TG 105 (Documentation provided to EFSA n. 7 and 10).

Two IBOs provided information on the LBG properties in water based on the scientific literature (Documentation provided to EFSA n. 9 and 11). The Panel noted that the key information was already included in the EFSA ANS Panel opinion from 2017 (EFSA ANS Panel, 2017). One IBO further stated that LBG with approximately 300 kDa is incompletely dispersed in water at room temperature and reaches full dispersibility at 86–89°C under 10 min stirring. The IBO further noted that a recommended practice in the food processing industry is to heat locust bean dispersions up to 95°C (Documentation provided to EFSA n. 11).

Additionally, one IBO provided information on the relationship between the concentration of LBG (E 410) and its viscosity in water (Documentation provided to EFSA n.9). The analysis was performed on three E 410 batches at different concentrations from 15 up to 33.3 g/L at 23–25°C demonstrating a linear relationship between concentration and viscosity of E 410.

The Panel noted that based on the data provided by the IBOs no conclusion can be drawn on the solubility properties in water or on the presence of small particles including nanoparticles in E 410. Furthermore, the Panel noted that LBG swells in cold water, but heating is necessary for maximum solubility (EFSA ANS Panel, 2017) and that as a hydrophilic macromolecule, in hot water it forms a colloidal dispersion.

### 3.2.7. Particle size

Upon request from EFSA, information on the particle size distribution of E 410 was provided. (Documentation provided to EFSA n. 7). One IBO provided information on particle size distribution of 10 batches of E 410 products determined by laser diffraction (LD) in a dry state. The IBO provided the information on particle size distribution by volume. One sample that showed the highest volume of fine particles was also analysed in a suspension with isopropyl alcohol. In all analysed samples, the size of measured particles was in the range of 0.4–1800 µm and the volume of particles below 550 nm was in the range of 0 - 0.36 % (differential volume).

The Panel noted that LD analysis is not considered a proper method to investigate the presence of nanosized particles as it does not provide information on the size of the constituent particles as required by the EFSA Scientific Committee Guidance on Technical requirements (EFSA SC Guidance-TR, EFSA Scientific Committee, 2021) and is prone to bias for polydisperse materials (Rauscher et al., 2019; Mech et al., 2020a,b).

The IBOs stated that they encountered serious technical challenges with identifying a suitable method for the particle size characterisation of E 410 according to the requirements of the EFSA SC Guidance -TR (Documentation provided to EFSA n. 8, 10, 11, 17).

The Panel acknowledges the IBOs' difficulty and stresses that currently no standardised methods are available for the polysaccharide thickening and gelling agents used as food additives, such as LBG (E 410) to measure the particle size distribution.

Based on the data on particle size distribution submitted by the IBO and the criteria set in the relevant EFSA SC Guidance -TR (EFSA Scientific Committee, 2021), the Panel concluded that in this specific case the presence of small particles, including nanoparticles, cannot be confirmed or excluded in the pristine food additive.

## 3.3. Exposure assessment

### 3.3.1. Authorised uses and use levels

Maximum levels of LBG (E 410) in foods for infants below 16 weeks of age are defined in Regulation (EC) No 1333/2008 on food additives, as amended. In this opinion, these levels are termed maximum permitted levels (MPLs).

According to Annex II to Regulation (EC) No 1333/2008, LBG (E 410) is authorised in 'dietary foods for infants for special medical purposes and special formulae for infants' (FC 13.1.5.1) and in 'dietary foods for babies and young children for special medical purposes' as defined in Directive 1999/21/EC (FC 13.1.5.2), see Table 6.

**Table 6:** MPLs of LBG (E 410) in foods for infants below 16 weeks of age according to Annex II to Regulation No 1333/2008

Food category number	Food category name	E number	Restrictions/exceptions	MPL (mg/l or mg/kg as appropriate)
13.1.5.1	Dietary foods for infants for special medical purposes and special formulae for infants	E 410	From birth onwards in products for reduction of gastro-oesophageal reflux	10,000
13.1.5.1	Dietary foods for infants for special medical purposes and special formulae for infants	E 410	(a)	1,000 <sup>(b)</sup>
13.1.5.2	Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC	E 410	From birth onwards in products for reduction of gastro-oesophageal reflux	10,000
13.1.5.2	Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC	E 410	(a)	1,000 <sup>(b)</sup>
13.1.5.2	Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC	E 410	Only gluten-free cereal-based foods <sup>(c)</sup>	20,000 <sup>(d)</sup>

Food category number	Food category name	E number	Restrictions/exceptions	MPL (mg/l or mg/kg as appropriate)
13.1.5.2	Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC	E 410	Only processed cereal based foods and baby foods <sup>(c)</sup>	10,000 <sup>(d)</sup>

MPL: maximum permitted level; LBG: locust bean gum.

(a): From its authorisation for FC 13.1.2.

(b): If more than one of the substances carrageenan (E 407), locust bean gum (E 410) and guar gum (E 412) is added to a foodstuff, the maximum level established for that foodstuff for each of those substances is lowered with that relative part as is present of the other substances together in that foodstuff.

(c): From its authorisation according to FC 13.1.3.

(d): Locust bean gum (E 410), guar gum (E 412), acacia gum (E 414), xanthan gum (E 415) and pectins (E 440) are authorised according to FC 13.1.3 individually or in combination.

LBG (E 410) is not authorised to be added in nutrients intended to be used in foodstuffs for infants and young children according to Annex III, Part 5, Section B to Regulation N°1333/2008.

### 3.3.2. Exposure data

Some food additives are authorised in the EU in infant formulae as defined by Commission Delegated Regulation (EU) 2016/127/EC (FC 13.1.1) and in dietary foods for infants for special medical purposes and special formulae for infants (FC 13.1.5.1) 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.

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<sup>14</sup> for technical and toxicological data on LBG (E 410) as a food additive for uses in foods for all population groups including infants below 16 weeks of age. In response to this public call, information on the actual use levels of LBG (E 410) in foods was made available to EFSA by industry. No analytical data on the concentration of LBG (E 410) in foods were made available by the Member States.

#### 3.3.2.1. Reported use levels in food category 13.1.5.1

One IBO provided EFSA with 14 use levels of LBG (E 410) (Documentation provided to EFSA n. 3,4). According to the same IBO, typical levels of LBG (E 410) are on average of 4,347 mg/L (from 650 to 10,000 mg/kg) and the highest level as reported by industry is 10,000 mg/L in infant formulae (FC 13.1.5.1) for infants below 16 weeks of age. The lowest use levels of LBG (E 410) provided were indicated as used in combination with other additives (pectins E 440).

#### 3.3.2.2. 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 4.1 million food and beverage products of which more than 1,300,000 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.<sup>15</sup>

For the purpose of this Scientific Opinion, Mintel's GNPD<sup>16</sup> was used for checking the labelling of food and beverage products and food supplements for LBG (E 410) within the EU's food market as the database contains the compulsory ingredient information on the label.

According to Mintel's GNPD, LBG (E 410) was labelled on nine products of 'Baby formula (0-6 months)' between January 2017 and November 2022, which represent less than 5% of the total number of food products belonging to this subcategory. These products are labelled as anti-reflux/anti-regurgitation/digestive disorders formulae, for babies from 0 to 36 months.

<sup>14</sup> Call for technical and toxicological data on locust bean gum (E 410) as a food additive for uses in foods for all population groups including infants below 16 weeks of age. Published: 18 July 2018. Available from: [https://www.efsa.europa.eu/sites/default/files/consultation/callsfordata/180718\\_locust.pdf](https://www.efsa.europa.eu/sites/default/files/consultation/callsfordata/180718_locust.pdf)

<sup>15</sup> Missing Cyprus, Luxembourg and Malta.

<sup>16</sup> <http://www.gnpd.com/sinatra/home/> accessed on 25/11/2022.

### 3.3.3. Exposure estimates

#### 3.3.3.1. Exposure estimates for infants below 16 weeks

Exposure to LBG (E 410) from its uses as a food additive in formulae for infants below 16 weeks was estimated. This scenario is based on the recommended consumption levels from SC Guidance (EFSA Scientific Committee, 2017). This guidance 'recommends values of 200 and 260 mL formula<sup>17</sup>/kg bw per day as conservative mean and high-level consumption values to be used for performing the risk assessments of substances which do not accumulate in the body present in food intended for infants below 16 weeks of age'. These recommended consumption levels correspond to 14–27 days old infants consumption. For the regulatory maximum level exposure assessment scenario, MPL for infant FSMP (10,000 for FC 13.1.5.1) was used. The maximum level provided by industry (equals to the MPL) and the average typical level (4,347 mg/kg) were used for the refined scenarios.

#### 3.3.3.2. Dietary exposure to LBG (E 410) from infant formulae

Table 7 summarises the estimated exposure to LBG (E 410) from its use as a food additive in FC 13.1.5.1 for infants below 16 weeks of age.

**Table 7:** Dietary exposure to LBG (E 410) in infant formulae (FC 13.1.5.1) for infants below 16 weeks of age according to Annex II to Regulation (EC) No 1333/2008 (in mg/kg bw per day)

	Infants (< 16 weeks of age)
<b>Regulatory maximum level exposure assessment scenario (10,000 mg/kg)</b>	
• Mean consumption (200 mL/kg bw per day)	2,000
• High-level consumption (95th percentile, 260 mL/kg bw per day)	2,600
<b>Refined estimated exposure assessment scenario</b>	
<b>Scenario using maximum use level reported by industry (10,000 mg/kg)</b>	
• Mean consumption (200 mL/kg bw per day)	2,000
• High-level consumption (95th percentile, 260 mL/kg bw per day)	2,600
<b>Scenario using mean of use levels reported by industry (4,347 mg/kg)</b>	
• Mean consumption (200 mL/kg bw per day)	869
• High-level consumption (95th percentile, 260 mL/kg bw per day)	1,130

LBG: locust bean gum; bw: body weight.

#### 3.3.4. Exposure estimates for infants above 16 weeks of age and toddlers consuming FSMP

As LBG (E 410) is authorised in the food categories 13.1.5.1 and 13.1.5.2, an additional exposure assessment scenario considering these two food categories was performed to estimate the exposure of infants (above 16 weeks) and toddlers (classified as young children in Commission Delegated Regulation (EU) 2016/127, 1–3 years old) who may eat and drink these FSMPs. The consumption of these foods is not reported in the EFSA Comprehensive database. To consider potential exposure to LBG (E 410) via these foods, the Panel assumes that the amount of FSMP consumed by infants and toddlers resembles that of comparable foods in infants and toddlers from the general population. Thus, the consumption of FSMP categorised as FC 13.1.5 was assumed equal to that of formulae and food products categorised as FCs 13.1.1, 13.1.2, 13.1.3 and 13.1.4. Use levels of LBG (E 410) for FC 13.1.5.2 were received in 2014 following a call for data<sup>18</sup> and used in the 2017 ANS Panel opinion (EFSA ANS Panel, 2017). Use levels for FC 13.1.5.1 were received in 2020 (see Section 3.3.2.1) and were used in this opinion. This scenario was estimated as follows:

- Consumers only of FSMP were assumed to be exposed to LBG (E 410) present at the maximum reported use level on a daily basis via consumption of FC 13.1.5.1 and FC 13.1.5.2. For the remaining food categories, the mean of the typical reported use levels was used.

<sup>17</sup> The term 'formula' has been added.

<sup>18</sup> <https://www.efsa.europa.eu/en/consultations/call/140309>

**Table 8:** Dietary exposure to LBG (E 410) for consumers only of FSMP, for infants above 16 weeks of age and toddlers, considering their whole diet, according to Annex II to Regulation (EC) No 1333/2008 (in mg/kg bw per day)

	Infants (> 16 weeks-1 year)	Toddlers (12–35 months)
<b>FSMP consumers only scenario</b>		
• Mean	376–1,107	122–653
• 95th percentile	1,077–1,653	541–1,225

LBG: locust bean gum; FSMP: food for special medical purposes; bw: body weight.

### 3.3.5. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainty have been considered and summarised in Table 9.

**Table 9:** Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction <sup>(a)</sup>
Consumption data for infants below 16 weeks of age: one reference point only to estimate exposure during the period of up to 16 weeks of age	+/-
Consumption data for infants above 16 weeks of age and toddlers:	+/-
• consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard for subjects above 16 weeks of age	
• Methodology used to estimate high percentiles (95th) long-term (chronic) exposure based on data from food consumption surveys covering only a few days for subjects above 16 weeks of age	+
• Correspondence of reported use levels to the food items in the EFSA Comprehensive Database: uncertainties to which types of food the levels refer	+/-
• Uncertainty in possible national differences in use levels of food categories	+/-
Regulatory maximum level exposure assessment scenario for infants below 16 weeks of age:	+
• exposure calculations based on the MPL according to Annex II to Regulation (EC) No 1333/2008	
Refined exposure assessment scenarios for infants below 16 weeks of age:	+
• exposure calculations based on the maximum levels (reported use from industries)	+/-
• exposure calculations based on the typical levels (reported use from industries)	
Refined exposure assessment scenarios for infants above 16 weeks of age:	+/-
• exposure calculations based on the maximum level for FC 13.1.5.1, for the remaining FCs the average of the typical level was considered	
Refined exposure assessment scenarios for toddlers:	+/-
• exposure calculations based on the maximum level for FC 13.1.5.2, for the remaining FCs the average of the typical level was considered	

MPL: maximum permitted level.

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

LBG (E 410) is authorised in food categories 13.1.5.1 and 13.1.5.2 according to Annex II to Regulation (EC) No 1333/2008.

There is uncertainty around the consumption values used and the typical use levels reported by industry and used in the calculations, since the actual formula consumption and related additive use levels in that formula could be higher or lower than the fixed values used in the scenario(s). This gives rise to the potential both for possible over- and under-estimation of exposure (+/- in Table 9). The actual use levels should not be higher than the MPL (so there is no uncertainty in that direction that could give rise to a potential for overestimation of exposure) but actual use levels (and so exposure)

could be lower. The consequence of this one-sided uncertainty is that assuming use levels are all at the MPL has the potential to overestimate exposure (+, Table 9).

Based on the assumption that carers of children would be brand-loyal to an infant formula for special medical purposes and considering that the maximum reported levels were used for foods under FC 13.1.5.1 and 13.1.5.2 while mean reported use levels were used for the rest of the diet, the Panel considered that the dietary exposure to LBG (E 410) would result in a realistic estimation of the exposure to LBG (E 410) from their uses as food additives according to Annex II for infants above 16 weeks of age and toddlers consuming FSMP (see Table 9).

### 3.4. Proposed revision to existing EU specifications for LBG E 410

The potential exposure to impurities from the use of the food additive E 410 can be calculated by assuming that the impurity is present in the food additive up to a limit value and then by calculation pro-rata to the estimates of exposure to the food additive itself.

In the current opinion, the dietary exposure for infants below 16 weeks of age and above 16 weeks to 3 years of age were estimated (see Table 7). For infants below 16 weeks of age, the refined exposure estimates based on the maximum and mean use levels of E 410 reported by industry (Table 7) were considered. The mean and 95th percentile exposure estimates were 2,000 and 2,600 mg/kg bw per day for the maximum use level and 839 and 1,130 mg/kg bw per day for the mean use level reported. For the infants above 16 weeks and toddlers, the dietary exposure to LBG (E 410) for consumers only of FSMP was calculated at the maximum use level (Table 8) reported by the industry and the highest mean and 95th percentile estimates were 1,107 and 1,653 mg/kg bw per day for infants from 16 weeks to 1 year of age, respectively.

With regard to the dietary exposure to the food additive for the general population, the Panel considered the exposure calculations for E 410 as presented in the re-evaluation of the food additive (EFSA ANS Panel, 2017). The Panel selected as the worse-case for all age groups the highest mean and 95th percentile which was calculated for toddlers to be 237 and 435 mg/kg bw per day respectively (EFSA ANS Panel, 2017).

The level of the impurity in the food additive combined with the estimated or potential intakes of E 410, as explained above could result in an exposure which can be compared with the following RPs or health-based guidance values (HBGVs) (Table 10) for the undesirable impurities potentially present in E 410.

**Table 10:** Reference points/health-based guidance values for impurities potentially present in E 410

Impurity/constituent/ HBGV/RP (ug/kg bw)	Basis/Reference
Lead (Pb)/ 0.5 (BMDL <sub>01</sub> )	The reference point is based on a study demonstrating perturbation of intellectual development in children with the critical response size of 1 point reduction in IQ. The EFSA CONTAM Panel mentioned that a 1 point reduction in IQ is related to a 4.5% increase in the risk of failure to graduate from high school and that a 1 point reduction in IQ in children can be associated with a decrease of later productivity of about 2%. A risk cannot be excluded if the exposure exceeds the BMDL <sub>01</sub> (MOE lower than 1). EFSA CONTAM Panel (2010)
Mercury (Hg)/ 4 (TWI)	The HBGV was set using kidney weight changes in male rats as the pivotal effect. Based on the BMDL <sub>10</sub> of 0.06 mg/kg bw per day, expressed as mercury, and an uncertainty factor of 100 to account for inter and intra species differences, with conversion to a weekly basis and rounding to one significant figure, a TWI for inorganic mercury of 4 µg/kg bw per week, expressed as mercury was established. EFSA CONTAM Panel (2012)
Cadmium (Cd)/ 2.5 (TWI)	The derivation of the reference point is based on a meta-analysis to evaluate the dose-response relationship between selected urinary cadmium and urinary beta-2-microglobulin as the biomarker of tubular damage recognised as the most useful biomarker in relation to tubular effects. A group-based BMDL <sub>5</sub> of 4 µg Cd/g creatinine for humans was derived. A chemical specific adjustment factor of 3.9 was applied to account for human variability in urinary cadmium within each dose-subgroup in the analysis resulting in a reference point of 1.0 µg Cd per g creatinine. In order to remain below 1 µg Cd/g creatinine in urine in 95 % of the population by

Impurity/constituent/HBGV/RP (ug/kg bw)	Basis/Reference
	age 50, the average daily dietary cadmium intake should not exceed 0.36 µg Cd/kg bw, corresponding to a weekly dietary intake of 2.5 µg Cd/kg bw. EFSA CONTAM Panel (2009a)
Arsenic (As)/0.3–8 (BMDL <sub>01</sub> )	The reference point is based on a range of benchmark dose lower confidence limit (BMDL <sub>01</sub> ) values between 0.3 and 8 µg/kg bw per day identified for cancers of the lung, skin and bladder, as well as skin lesions. In general, the MoE should be at least 10,000 if the reference point is based on carcinogenicity in animal studies. However, as the BMDL for As is derived from human studies, an interspecies extrapolation factor (i.e. 10) is not needed, i.e. a MoE of 1,000 would be sufficient. EFSA CONTAM Panel (2009b); EFSA Scientific Committee (2012)

HBGV: health-based guidance value; RP: reference point; BMDL<sub>01</sub>: benchmark dose (lower confidence limit); bw: body weight; TWI: Tolerable Weekly Intake; MoE: margin of exposure.

The risk assessment of the undesirable impurities helps inform whether there could be a possible health concern if these impurities would be present at the limit values in the food additive. The assessment is performed by calculating the margin of exposure (MoE) by dividing the RP (e.g. BMDL Table 10) by the exposure estimate (Tables 7, 8 and EFSA ANS Panel, 2017), or by estimating the contribution of the use of E 410 to the HBGV (expressed as percentage of the HBGV).

### 3.4.1. Toxic elements

The Panel noted that the occurrence data on toxic elements submitted by the IBOs are lower than the current limits in the EU specifications (Documentation provided to EFSA n. 1,2,3,5). The Panel considered that the maximum limits in the EU specifications for toxic elements should be established based on actual levels in the commercial food additive. If the European Commission decides to revise the current limits in the EU specifications, the estimates of toxic elements intake as presented below could be considered.

The Panel agreed that the lowest technologically achievable levels proposed by the IBO for the clarified LBG were consistent with the occurrence data on toxic elements that were submitted. However, the IBO proposal for the lowest achievable levels for toxic elements in the unclarified LBG is substantially higher than reported levels measured in commercial batches of unclarified E 410. For the elements Pb, Hg, Cd and As, the lowest technologically achievable levels proposed for the unclarified food additive were 0.5, 0.5, 0.25 and 1.5 mg/kg, respectively. For the clarified E 410 additive, the lowest technologically achievable levels values proposed were 1, 0.1, 0.1 and 0.3 mg/kg, respectively. Both proposals are lower than the current EC specifications for these toxic elements in the LBG E 410 which are not more than 2, 1, 1 and 3 mg/kg for Pb, Hg, Cd and As, respectively.

The Panel performed the risk assessment that would result if these toxic elements were present in E 410, at (i) the maximum current limit in the EU specification and (ii) at the lowest technologically achievable levels proposed by the IBOs for unclarified LBG E 410. Although one IBO stated that, to the best of its knowledge, only unclarified LBG is used in infant formulae (FSMP), another IBO stated that from a technical point of view, clarified LBG can replace unclarified LBG in all food applications. For this reason, the calculations (iii) are also presented using the proposed lowest technologically achievable levels for the clarified LBG for the purpose of illustration.

The Panel emphasised that the choice of the maximum limits for toxic elements in the specifications is in the remit of risk management. The numbers used here are merely taken to support the risk assessment of these toxic elements as presented below.

The outcome of the risk assessment of the FAF Panel (Table 11) illustrates the health impact that could result if the maximum limits for toxic elements according to the current EU specifications (i) or revised maximum limits (ii, iii) were to be used.

**Table 11:** Risk assessment for toxic elements

<b>(i) Based on the current EU specifications limits for toxic elements in E 410 for use in food for all age groups</b>				
<b>Exposure to E 410 (mg/kg bw per day)</b>	<b>MoE for Pb at 2 mg/kg</b>	<b>% of the TWI for Hg at 1 mg/kg</b>	<b>% of the TWI for Cd at 1 mg/kg</b>	<b>MoE for As at 3 mg/kg</b>
2,000 <sup>(a)</sup>	0.125	350%	560%	0.05–1.35
2,600 <sup>(b)</sup>	0.10	460%	730%	0.04–1.03
869 <sup>(c)</sup>	0.29	150%	240%	0.12–3.07
1,130 <sup>(d)</sup>	0.22	200%	320%	0.09–2.36
1,107 <sup>(e)</sup>	0.23	194%	310%	0.09–2.41
1,653 <sup>(f)</sup>	0.15	289%	463%	0.06–1.61
237 <sup>(g)</sup>	1.05	42%	66%	0.42–11.2
435 <sup>(h)</sup>	0.57	76%	120%	0.23–6.2
<b>(ii) Based on the lowest technologically achievable levels in unclarified E 410 for use in food for all age groups as proposed by the interested business operator (Documentation provided to EFSA n. 1 and 3)</b>				
<b>Exposure to E 410 (mg/kg bw per day)</b>	<b>MoE for Pb at 0.5 mg/kg</b>	<b>% of the TWI for Hg at 0.5 mg/kg</b>	<b>% of the TWI for Cd at 0.25 mg/kg</b>	<b>MoE for As at 1.5 mg/kg</b>
2,000 <sup>(a)</sup>	0.50	175%	140%	0.10–2.67
2,600 <sup>(b)</sup>	0.38	228%	182%	0.08–2.05
869 <sup>(c)</sup>	1.15	76%	61%	0.23–6.14
1,130 <sup>(d)</sup>	0.88	99%	79%	0.18–4.72
1,107 <sup>(e)</sup>	0.9	97%	77%	0.18–4.82
1,653 <sup>(f)</sup>	0.6	145%	116%	0.12–3.23
237 <sup>(g)</sup>	4.22	21%	17%	0.84–22.5
435 <sup>(h)</sup>	2.30	38%	30%	0.46–12.3
<b>(iii) Based on the lowest technologically achievable levels in clarified E 410 for use in food for all age groups as proposed by the interested business operator (Documentation provided to EFSA n. 5)</b>				
<b>Exposure to E 410 (mg/kg bw per day)</b>	<b>MoE for Pb at 1.0 mg/kg</b>	<b>% of the TWI for Hg at 0.1 mg/kg</b>	<b>% of the TWI for Cd at 0.1 mg/kg</b>	<b>MoE for As at 0.3 mg/kg</b>
2,000 <sup>(a)</sup>	0.25	35%	56%	0.50–13.3
2,600 <sup>(b)</sup>	0.19	46%	73%	0.38–10.3
869 <sup>(c)</sup>	0.58	15%	24%	1.2–31
1,130 <sup>(d)</sup>	0.44	20%	32%	0.88–24
1,107 <sup>(e)</sup>	0.45	19%	31%	0.9–24.1
1,653 <sup>(f)</sup>	0.3	29%	46%	0.6–16.1
237 <sup>(g)</sup>	2.11	4.2%	6.6%	4.2–113
435 <sup>(h)</sup>	1.15	7.6%	12%	2.3–61

bw: body weight; TWI: Tolerable Weekly Intake; MoE: margin of exposure.

- (a): Mean exposure level for the population below 16 weeks of age (Refined estimated exposure assessment scenario using the maximum use level reported by industry (10,000 mg/kg) in infant FSMP (FC 13.1.5.1)).
- (b): 95th percentile exposure level for the population below 16 weeks of age (Refined estimated exposure assessment scenario using the maximum use level reported by industry (10,000 mg/kg) in infant FSMP (FC 13.1.5.1)).
- (c): Mean exposure level for the population below 16 weeks of age (Refined estimated exposure assessment scenario using the mean of use levels reported by industry (4,347 mg/kg) in infant FSMP (FC 13.1.5.1)).
- (d): 95th percentile exposure level for the population below 16 weeks of age (Refined estimated exposure assessment scenario using the mean of use levels reported by industry (4,347 mg/kg) in infant FSMP (FC 13.1.5.1)).
- (e): Highest exposure level for the population above 16 weeks age and toddlers (Refined estimated exposure assessment scenario using the maximum use levels reported by industry (10,000 mg/kg) in infant FSMP (FC 13.1.5.1) – infants above 16 weeks to 1 year, - mean).
- (f): Highest exposure level for the population above 16 weeks age and toddlers (Refined estimated exposure assessment scenario using the maximum use levels reported by industry (10,000 mg/kg) in infant FSMP (FC 13.1.5.1) – infants above 16 weeks to 1 year -95th percentile).

- (g): Highest exposure level for the general population (Refined Brand-Loyal Scenario -Toddlers – mean (data from, Section 3.4.1.3, EFSA ANS Panel, 2017 on E 410)).
- (h): Highest exposure level for the general population (Refined Brand-Loyal Scenario -Toddlers – 95th percentile (data Section 3.4.1.3, EFSA ANS Panel, 2017 on E 410)).

The resulting figures show that the potential for exposure to toxic elements from the consumption of E 410 could be substantial.

The Panel noted that the MoE values for arsenic are not sufficient both for the current EU specifications as well as for the specifications proposed by the IBOs. In the case of lead the MoE values are not sufficient for all age groups with the exemption of the general population when applying the lowest achievable levels proposed by the IBOs. The assessment of the uncertainty in the exposure showed no potential for overestimation of exposure for the different scenarios.

The Panel noted that by applying the lowest achievable levels proposed by the IBOs for mercury and cadmium, exposures to these toxic elements from the use of clarified LBG could represent a significant fraction of their TWI values. Furthermore, the use of unclarified LBG could lead to exceedances of the TWI values for most age groups (see Table 11). The existing maximum limits for Hg and Cd in E 410 EU specifications will lead to exceedance of the TWIs for these elements (see Table 11).

The Panel agreed that the calculations indicate the need to decrease the current maximum limits for Pb, Hg, Cd and As in E 410, considering also other sources of exposure to these toxic elements. Furthermore, the Panel noted that maximum levels for lead and cadmium in infant formula are set by Reg. (EC) No 1881/2006<sup>19</sup>; therefore, the Panel calculated the impact of the level of the toxic elements lead and cadmium in the food additive on the final product and compared that with the legal limits for these elements in the final formula for the infants below 16 weeks of age (see Appendix E). Considering the results of these estimations and the fact that the food additive is not the only potential source of toxic elements in the infant formula the Panel emphasises the need to reduce the specification limit values for lead and cadmium in Regulation (EU) no 231/2012.

### 3.4.2. Other parameters

The Panel noted that JECFA has separate specifications for LBG and clarified LBG (Table 1) and that one IBO supported this distinction and proposed specifications specifically for clarified LBG (Table 5). Based on the newly provided data and the Panel's considerations (see also Sections 3.2, 3.5.2.3 and 3.5.3), the Panel considered that the current level in proteins in unclarified LBG (E 410) does not raise a specific concern for allergenicity. Therefore, the Panel does not see the need to introduce separate specifications for unclarified and clarified LBG E 410 (see discussion in Section 3.6).

Because of both the botanical origin and the polysaccharidic nature of LBG, it can be a substrate prone to microbiological contamination. Therefore, the Panel is of the view that microbiological specifications should be introduced in line with the specifications of JECFA (see Table 1). The microbiological specifications should also include *Cronobacter (Enterobacter) sakazakii*, set on the basis of the information provided.

Overall, based on the analytical data provided by the IBOs in response to the EFSA call for data<sup>8</sup> (Documentation provided to EFSA n. 1, 2, 3, 5) and the above considerations, the Panel recommends the revisions of the existing EU specifications for LBG E 410 as listed in Table 12.

The Panel noted that E 410 is a hydrophilic macromolecule which in hot water forms a colloidal dispersion in which the macromolecules and/or polymolecular particles are dispersed throughout the liquids (liquid formulations, physiological fluids in the GI-tract). They are not forming true solutions (molecular disperse systems) and are specific for their gelling properties. Therefore, the Panel recommends changing the word 'soluble' to 'fully dispersible' in the EU specifications of E 410 (see Table 12).

The Panel also considered that the CAS number 9000-40-2 corresponding to locust bean gum should be included in the existing EU specifications for E 410.

<sup>19</sup> Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (Text with EEA relevance) OJ L 364, 20.12.2006, pp. 5–24, consolidated version: 1/7/2022.

**Table 12:** Proposal for a revised version of the existing EU Specifications for locust bean gum (E 410)

	Commission Regulation (EU) No 231/2012	Comment/justification for revision
Definition	See Table 1	Unchanged
CAS number	–	To be included 'CAS Number 9000-40-2'
Molecular weight	See Table 1	Unchanged
Assay	See Table 1	Unchanged
Description	See Table 1	Unchanged
<b>Identification</b>		
Test for galactose	See Table 1	Unchanged
Test for mannose	See Table 1	Unchanged
Solubility	Soluble in hot water, insoluble in ethanol	To be revised 'Fully dispersible in hot water, insoluble in ethanol'
Microscopic examination	See Table 1	Unchanged
<b>Purity</b>		
Loss on drying	See Table 1	Unchanged
Ash	See Table 1	Unchanged
Acid-insoluble matter	See Table 1	Unchanged
<b>Protein (N × 6.25)</b>	See Table 1	Unchanged
Starch	See Table 1	Unchanged
Lead	Not more than 2 mg/kg	Lowered on the basis of the information provided and on the considerations of the Panel
Arsenic	Not more than 3 mg/kg	Lowered on the basis of the information provided and on the considerations of the Panel
Mercury	Not more than 1 mg/kg	Lowered on the basis of the information provided and on the considerations of the Panel
Cadmium	Not more than 1 mg/kg	Lowered on the basis of the information provided and on the considerations of the Panel
Ethanol and propan-2-ol	Not more than 1%, single or in combination	Unchanged
<b>Microbiological criteria</b>		
Aerobic plate count	Not presently specified	Microbiological criteria should be included in line with JECFA specifications
Total yeasts and moulds	Not presently specified	Microbiological criteria should be included in line with JECFA specifications
<i>E. coli</i>	Not presently specified	Microbiological criteria should be included in line with JECFA specifications
<i>Salmonella</i> spp.	Not presently specified	Microbiological criteria should be included in line with JECFA specifications
<i>Cronobacter (Enterobacter) sakazakii</i>	Not presently specified	Microbiological criteria should be included on the basis of the information provided

### 3.5. Biological and toxicological data

#### 3.5.1. Previous evaluation by ANS Panel (2017)

The following text (in italics) is from the opinion published in 2017 (EFSA ANS Panel, 2017). New information and assessments related to the specific age group below 16 weeks of age are added in the following paragraph.

#### **Absorption, distribution, metabolism and excretion**

*There is evidence that certain high molecular weight dietary polysaccharides, such as gums, could be partially broken down in the large intestine of man. In addition to intermediate metabolites, such as lactate, acrylate or fumarate, the main end products of this colonic anaerobic digestive process are*

short-chain fatty acids (SCFAs), such as acetic, propionic and butyric acids, which are absorbed from the colon (Cummings and Englyst, 1987). Overall, data on *in vitro* degradation by human gastrointestinal fluids and on *in vivo* digestibility of locust bean gum in animals demonstrated that this compound would not be absorbed intact or hydrolysed by digestive enzymes. However, locust bean gum would be fermented with production of SCFAs, such as acetic, propionic and butyric acids, during its passage through the large intestine by strains of bacteria found in the human colon. Based on the available knowledge on the role of SCFA as end products of the fermentation of dietary fibres by the anaerobic intestinal microflora (Topping and Clifton, 2001; den Besten et al., 2013), the Panel considered that their potential formation as fermentation products from locust bean gum does not raise any concern. Despite the absence of convincing *in vivo* study in humans, the Panel considered that these data indicate that locust bean gum would most probably not be absorbed intact but significantly fermented by enteric bacteria in humans.

### Acute, subchronic, genotoxicity, chronic, developmental and reproductive toxicity studies

Locust bean gum is regarded as not acutely toxic, based on the results of acute oral toxicity studies. Three subacute toxicity studies (Ershoff and Wells, 1962; Vohra et al., 1979; Mallett et al., 1984) have been described in rats. In a subchronic toxicity study in rats by Til et al. (1974), no adverse effects were reported at doses up to 4,500 mg of locust bean gum/kg bw per day. In the NTP (1982), no adverse effects were reported in mice and rats receiving doses up to 20,000 and 9,000 mg of locust bean gum/kg bw per day, respectively. The Panel noted that the NTP study did not include haematology, urinalysis and clinical chemistry.

The available *in vitro* and *in vivo* data for genotoxicity were limited. However, no genotoxic activity was observed for locust bean gum. In addition, considering the chemical structure of locust bean gum and its negligible absorption the Panel concluded that there is no concern with respect to the genotoxicity of locust bean gum (E 410).

Locust bean gum was tested for carcinogenicity in mice and rats receiving doses up to 7,500 mg of locust bean gum/kg bw per day up to 2,500 mg of locust bean gum/kg bw per day, respectively, for 103 weeks (Carlson and Domanski, 1980 as reported by JECFA; NTP, 1982; Melnick et al., 1983). The Panel noted that in these studies, locust bean gum was not carcinogenic.

The Panel considered that no adverse effects were reported in a three-generation reproductive toxicity study receiving doses up to 5% locust bean gum in the diet (equivalent to 4,500 mg of locust bean gum/kg bw per day), the highest dose tested. In the prenatal developmental toxicity studies in rats and hamsters, no maternal and developmental effects were observed up to the highest dose (1,300 and 1,000 mg locust bean gum/kg bw per day) (FDRL, 1972).

### Human data- infants and children

Locust bean gum is described to be frequently added to infant formulae in the treatment of noncomplicated gastro-oesophageal reflux (GOR) (Meunier et al., 2014). The efficacy of thickening agents depends on their ability to increase gastric retention time, avoiding a return to the oesophagus during the first digestion phase (Corvaglia et al., 2013). These types of products are commercialised under the name of antireflux or antiregurgitation infant formulae (AR formulae), and are promoted with the claim that they benefit infants who have GOR or who spit up regularly. The North American and the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN and ESPGHAN, respectively) commented on the use of AR formulae in physiological GOR and gastro-oesophageal reflux disease (GORD) and reported that it may decrease visible regurgitation but does not result in a measurable decrease in the frequency of oesophageal reflux episodes. However, long-term use would require additional studies (Vandenplas and Rudolph, 2009). Authors of a systematic review concluded that there was no evidence from randomised controlled trials to support or refute the efficacy of feed thickeners in newborn infants with GOR (Huang et al., 2002). Locust bean gum as ingredient for AR formulae, is legally allowed in Europe. Locust bean gum may be added up to a maximum level of 10 g/L from birth onwards (see Section 3.2). As previously mentioned, locust bean gum was considered by the SCF as acceptable for its use up to 1 g/100 mL (10 g/L) in Foods for Special Medical Purposes when prescribed under medical supervision to treat GORD (SCF, 2003) (see Section 1.2).

### Paediatric case reports

Few cases of severe adverse effects or fatality in premature or low birth weight infants with GORD receiving infant formulae thickened with locust bean gum were reported in the open literature. The

origin of such effects could be related to the very immature state of their gastrointestinal tract. Sievers and Schaub (2003) reported a study in which six premature infants (age not specified) were fed 0.2–0.5 g/100 mL of locust bean gum formula to reduce vomiting. This treatment resulted in an increased frequency of defaecation, metabolic acidosis and hypokalaemia as long as the exposure continued. Clarke and Robinson (2003) reported fatal necrotising enterocolitis observed on days 26 and 30, in two extremely low birth weight premature infants fed locust bean gum thickened milk (dose unspecified). According to the authors, thickened milk may have led to a bowel obstruction resulting in a necrotising enterocolitis; however, the pathophysiology of these two cases was not investigated because no post-mortem examination was done. A study was conducted in 166 bottle-fed infants under 4 months of age, who were presented with frequent regurgitation/vomiting due to uncomplicated GOR at the outpatient clinics of six paediatric centres (Iacono et al., 2002). Patients were divided into two groups. Group 1 comprised 82 infants (45 males, 37 females; median age 1.5 months) who were treated with an AR formula available on the Italian market thickened with locust bean gum (the concentration is not given, but is expected to not exceed MPLs according to the EU food legislation). Group 2 consisted of 84 patients (43 males, 41 females; median age 1.5 months) treated with a common, adapted formula without any thickening component. No differences between the two groups in regurgitation scores were recorded either at baseline, after 4 and 8 weeks of treatment. But in 14 patients in the group receiving the formula thickened with locust bean gum, treatment was suspended due to the onset of diarrhoea during the first 2 weeks of the study. The authors concluded that the use of the thickened formula could cause diarrhoea.

Considering the specific group of infants of more than 12 weeks of age, a case of hypersensitivity and cases of gastrointestinal discomfort have been associated with the use of locust bean gum in infant formulae to treat GOR and are described in the following. An immediate hypersensitivity to locust bean gum after oral ingestion has been reported in an infant (Savino et al., 1999). The report described a 5-month-old girl, suffering from GOR who developed recurrent vomiting, urticaria and flushing after ingestion of a milk formula containing locust bean gum as a thickening agent. Diagnosis was based only on a case history but no specific tests were performed. Vivatvakin and Buachum (2003) described the effect of a locust bean gum milk formula (unspecified concentration) on gastric emptying and on regurgitation in 20 Thai infants (4–24 weeks old). Statistically significant improvements in symptoms of vomiting and in weight gain per week were observed in infants consuming the locust bean gum formula for 2–4 weeks. However, there was no significant difference in gastric emptying half time. The only side effect was a significantly increased flatus observed in one infant. Miyazawa et al. (2006) investigated the effects of milk-based formulae thickened with two different concentrations of locust bean gum (0.4 and 0.5 g/100 mL, formulae HL-350 and HL-450, respectively) on gastric emptying in 27 infants (18–19 weeks old) with recurrent regurgitation episodes. The thickened formula containing the higher concentration of locust bean gum (0.5 g/100 mL) slowed gastric emptying in infants with GOR. In this study, the mothers of two subjects fed with HL-350 and one subject fed with HL-450 reported an increase in bowel movements, but none of these infants had severe diarrhoea. The Panel noted that the highest applied dose reported was only half of the MPL of locust bean gum in formulae for special medical purposes (categories 13.1.5.1 and 13.1.5.2). Carré described that in the management of GOR of infants, carob seed (locust bean) preparations added to bottle feeds in the proportion of 1 g to 115 mL were given. The author indicated that the treatment could cause frequent loose gelatinous stools which might occasionally necessitate the temporary withdrawal of the treatment (Carré 1985).

### **Possible effects of locust bean gum on the bioavailability of nutrients**

Several authors have indicated the need to explore further the effect of thickening agents on the nutrition and health of infants as some studies in *in vitro* model have suggested that the bioavailability of calcium, iron and zinc may be affected by these compounds (Bosscher et al., 2000, 2003a,b; Vandenplas et al., 2011; Gonzalez-Bermudez et al., 2014). However, one *in vivo* human study (Behall et al., 1987) did not support this hypothesis.

### **Other studies including hypersensitivity, allergenicity and food intolerance**

In human adults, there are few case reports of allergy to locust bean gum after oral ingestion. However, most of the reports described rhinitis and asthma that were essentially caused by occupational contact with locust bean gum. The Panel recognised the possible potential allergenicity of locust bean gum; however, it considered that the specification and origin of the gum are lacking in these studies. Case reports of hypersensitivity reactions associated with locust bean gum included the

case of a 5-month-old infant [Savino et al., 1999], the Panel considered that this hypersensitivity might be due to the locust bean gum proteins and therefore their content should be reduced as much as possible. The Panel further noted that in a group of 28 hypercholesterolaemic or normal adolescents and adults treated with locust bean gum for 8 weeks, doses up to 500 mg/kg bw per day were well tolerated without side effects.

### 3.5.2. Newly available data

#### 3.5.2.1. Absorption, distribution, metabolism and excretion

No new data were available.

#### 3.5.2.2. Toxicological data

A study 'Locust Bean Gum Piglet Adapted Formula Study in Juvenile Minipig' was made available to the Panel (Ellemann-Laursen, 2020 and the final amended report No 1 of this study, 2022; documentation provided to EFSA n. 3,4,8). The design of this study followed the EMA (2009) and ICH (2010) guidelines, and the EFSA guidance (2017). The study was performed according to Good Laboratory Practice (GLP). In addition, a (non-GLP) report 'Microbiota Analysis report: Effect of Locust Bean Gum Thickener on the fecal microbiota composition in juvenile Gottingen Minipigs' was made available (Tims et al., reporting data from 2020 and additional information 2022; Documentation provided to EFSA n. 3,8).

The study in neonatal piglets (Ellemann-Laursen, 2020; 2022, Documentation provided to EFSA n. 3,4,8) was assessed by means of a risk of bias (RoB) scoring scheme and was allocated to tier 1 (low risk of bias), see Appendix B.

The study evaluated the acceptance, tolerability and safety of LBG dissolved in a sow's milk replacement fed to juvenile minipigs from postnatal day (PND) 7/8–9 for 21 consecutive days (until PND 28). Forty-eight juvenile minipigs (24 males and 24 females, Ellegaard Göttingen Minipigs A/S, DK-4261 Dalmose, Denmark) were delivered by 9 sows. The piglets were separated from the sows on PND 3/4 and fed a formula (sow's milk replacer containing whey protein) until PND 5/7. Not later than PND 6/7, the piglets were allocated to 4 groups of 6 males and 6 females, each. The allocation was performed considering even distribution of littermates between the groups and reduction of intergroup mean body weight differences. From PND 7/8–9, piglets received every third hour a formula added LBG at concentrations of 0 (control), 3.5, 5.0 or 8.0 g/L. The high concentration of 8 g LBG/L was, according to the authors, the highest dissolved concentration technically achievable in the piglet test formula. The mean daily LBG consumption, calculated based on the recorded formula intake from day 7/8–9 to PND 28 was 1,050, 1,500, or 2,400 mg/kg bw per day for the low-, mid- and high-dose groups, respectively.

All animals but one survived up to the termination; one male piglet from the high-dose group was euthanised prematurely on PND 22 due to continuous watery faeces, subdued behaviour and decreased formula consumption. This male was treated with antibiotics from dosing day 8 to 16. A Faecal sample taken on PND 18 demonstrated detectable concentrations of *Enterococci*, *Klebsiella pneumoniae*, *E. coli* and *Candida albicans*. Faecal samples taken at necropsy demonstrated presence of *Enterococci* and *Klebsiella pneumoniae*. Necropsy and microscopic examination revealed inflammatory changes in the caecum and colon.

No treatment-related adverse effects on clinical signs, formula consumption, urinalysis, and macroscopic and microscopic findings were seen in the animals surviving to the end of the experimental feeding.

Faeces consistency evaluation revealed a higher incidence of less hard stools and a lower incidence of loose stools, and absence of watery faeces in LBG treated groups. The decrease in loose stools appeared dose dependent. The number of hard stools was comparable between the control and LBG treated groups.

The body weight gain of LBG treated piglets was not statistically significantly increased compared to control in mid- and high dose males (+4% and +11%, respectively) and in low-, mid- and high-dose females (+3%, +4% and +4%, respectively).

The haematological investigation revealed several differences to control in red blood cell parameters: non statistically significant decreases in haemoglobin (–10%), haematocrit (–9%) in males and females along with increases in reticulocyte counts (+26% in males and 9% in females). These changes were seen at the high dose. In the treated females, the decreases in MCV (–9%), in MCH (–11%) and in MCHC (–3%) were statistically significant at the high dose. However, the effect

size was small. The Panel noted that the above changes could be related to decreases of zinc concentrations in the blood of LBG-treated piglets. However, the changes in red blood cell parameters were small and/or not statistically significant, and therefore, their toxicological relevance was considered to be unclear.

The clinical chemistry investigation revealed statistically significant increased concentrations of total protein (+8%) and globulin (+55%) in low-dose males and a decreased concentration of magnesium (−6%) in mid-dose females. The Panel considered the changes as not treatment related as there was no dose–response relationship. Glucose concentration was statistically significantly increased relative to the control in the mid- and high-dose females (+26% and 45%, respectively). The Panel noted that glucose concentrations in low-dose females (+6%) and in all treated males (+6%, +14%, +28% in low-, mid- and high-dose males, respectively) were also increased but the difference to the control did not reach statistical significance. Regarding the changes in glucose concentration the Panel noted that the blood samples were from non-fasted animals. This, however, could not explain the difference in glucose concentration between the control and treated groups. The Panel considered the increase in glucose concentration as treatment related and toxicologically relevant as there was a dose–response relationship and the changes were seen in both sexes, although in males not statistically significant compared to controls. The Panel further noted that increases in glucose levels might be related to the reduced zinc levels.

Analysis of vitamins, zinc and copper in the blood revealed no differences to control except for a dose dependent decrease in zinc concentration in all treated males (−10%, −16%, −28%, at the low-, mid- and high dose, respectively) and females (−6%, −18%, −23%). The difference to controls reached statistical significance in high-dose males and mid- and high-dose females. The Panel considered the changes in zinc concentration as treatment related.

Analysis of organ weights revealed dose-related increases in absolute and relative weights of jejunum, caecum, and colon of LBG-treated males and of duodenum and jejunum of treated females. The changes were statistically significant for absolute and relative weights of jejunum (+43% and +35%, respectively) and caecum (+44% and +36%, respectively) in high-dose males, for the absolute weight of colon (+49%) in high-dose males, for the absolute weight of jejunum in mid- and high-dose females (+28% and +45%, respectively) and for relative weights of duodenum (+57%) and of jejunum (+41%) in high-dose females.

Macroscopic and microscopic examination of organs and tissues collected at the scheduled termination (gallbladder, kidneys, liver, pancreas, large intestines: cecum, colon, rectum, duodenum, small intestines: duodenum, jejunum, ileum, distal ileum, Peyer's patches, stomach) did not reveal any adverse morphological changes. The Panel agreed with the authors that the increased weights of the GI tract segments in growing piglets can be explained by an effect of a fibre-rich diet, and in the absence of associated clinical signs or histopathological changes, the findings were considered of no toxicological relevance.

Overall, the Panel considered that clinical appearance, defecation, formula consumption, body weight and weight and morphology of small and large intestine were not adversely affected by the dietary treatment with LBG in doses amounting to 2,400 mg LBG/kg bw per day, the highest dose tested. The Panel noted, however, that at this dose changes in some red blood cell parameters were observed. A dose-related increase in blood glucose levels and a dose related decrease in blood zinc concentration were also reported. The Panel considered that these changes should not be ignored and decided to use these endpoints for BMD analysis with a benchmark dose response (BMR) of 20% for zinc and 30% for glucose (for details see the Section 3.5.4 and Appendixes C and D).

**Tims et al.** (reporting data from 2020 and additional information 2022; Documentation provided to EFSA n. 3,8) described the analysis of microbiota of the piglets of the study of Ellemann-Laursen (2020 and 2022). Faecal samples were taken before LBG was administered in the formula (PND 3–5,7 samples) and at the end of the administration period (PND 26–28) from all groups. Eleven, 13, 11 or 10 samples were available from the control, 3.5, 5.0 or 8.0 g LBG/L groups. No indication on the number of animals or the sex was presented. Fluorescence in situ hybridisation (FISH) was used to determine the bacterial composition of the faecal microbiota. The bacterial target groups and the associated probes used were: *Bifidobacterium* spp. (Bif164m); Subset Enterobacteriaceae (Ec1531); *Eubacterium rectale* and *Blautia coccooides* group (Erec482); and the *Lactobacillus–Enterococcus* group (Lab158). At the end of the administration period, no differences were seen for the four bacterial groups tested between the control and the LBG-treated groups. However, the entire microbiota cell count was increased in the high-dose group when compared to the control group. The authors

suggested that this increase is due to the additional availability of substrate for the gastrointestinal microbiota due to (partial) fermentation of LBG.

### 3.5.2.3. Clinical data

The IBO provided four clinical studies on formulae thickened with LBG for the dietary management of GOR (Documentation provided to EFSA n. 3).

The study of Tounian et al. (2020; Documentation provided to EFSA n. 3) was an observational, i.e. without control group, non-blinded, prospective multicentre study, including 2,604 infants from which 96% were younger than 4 months of age. The study was conducted between January 2015 and December 2016. After inclusion the infants were exclusively fed for 1 month with the formula thickened with 3% LBG (0.42 g/100 mL of reconstituted formula), further containing postbiotics<sup>20</sup> derived from the Lactofidus™ fermentation process with two bacterial strains *Bifidobacterium breve* C50 and *Streptococcus thermophilus*. Inclusion criteria were the following: infants full-term born, formula fed, and presenting symptoms of moderate-to-severe regurgitation according to parental perception. Two visits were performed: the first at inclusion, and the second at the end of the intervention period. A questionnaire to collect the required information was used at both visits. The questionnaire contained queries on defaecation frequency and consistency, frequency of episodes of gas production, and quality of life parameters (crying duration, sleep quality, behaviour in the infant, anxiousness of the parents). Seven per cent of the participants dropped out, in 3 % due to intolerance of the formula. In 8 infants, allergy to cow's milk protein was suspected. In the publication anthropometric parameters were not reported. Because the study had an observational design, without a control group, the obtained information is of very limited value. The pre-/post-comparisons presented by the authors are very likely not treatment but time related. No serious adverse events were reported.

The observational study of the 'evolution of digestive symptoms in a cohort of infants fed by infant formula containing locust bean gum' (TELIA2 study; Documentation provided to EFSA n. 3, 4) had a similar design as the study of Tounian et al. (2020). The study was uncontrolled and not blinded with 3 months duration. From the included 190 infants, who were aged 0–4 months, 50% were 1–2 months old. The study was conducted between November 2019 and January 2021. The infants were fed for 3 months with a formula thickened with locust bean gum (0.4 g/100 mL), containing prebiotics short-chain galacto-oligosaccharides (scGOS) and long chain fructo-oligosaccharides (lcFOS) (0.4 g/100 mL, 9:1 ratio) and further containing postbiotics derived from the Lactofidus™ fermentation process. From the 190 infants 7 were lost to follow-up. Twenty per cent of the infants (n = 37) had respiratory infections during the study. Twenty-two infants had adverse events during the study, 17 had not-serious gastrointestinal symptoms, the other symptoms/disease were not treatment related (varicella, pyelonephritis, pharyngitis, sleep disorder) with the exception of eczema which was reported to be possibly a symptom of cow milk's allergy. Anthropometric measurements for weight, length and head circumference were performed at Baseline, Month 1 and Month 3 in the study. There were no remarkable results. However, because of the absence of a control population the results are hard to evaluate. The Panel explored the possibility to compare the study results concerning growth with non-randomised comparisons (i.e. comparisons to historical controls, such as comparisons to growth reference charts the data of which are of observational nature), but considered that the available data on these growth reference charts were not sufficiently informative for the European population. Hence, from this study the contribution to the risk assessment of locust bean gum (E 410) is very limited.

The **Qualinov survey** (Documentation provided to EFSA n. 3) was an observational, prospective multicentre (n = 279 paediatricians) study, including 751 infants. The study was conducted between November 2008 and July 2009. After inclusion, the infants were fed for 4 weeks with a different formula thickened with locust bean gum and starch, the content of which is not mentioned in the document submitted to EFSA. Inclusion criteria were as follows: infants <6 months, fed exclusively with an infant formula, with no plans of introduction to complementary feeding for the upcoming month; infants seen in consultation for simple regurgitations; and parents agreeing to come for a follow-up visit. Criteria for not including an infant in the study were at least one of the followings: breastfed at inclusion, premature, low birth weight, kept in nursery, height and weight delay, symptoms suggestive of esophagitis, intolerance to cow's milk protein, anti-reflux medication(s), chronic digestive, neurological or renal underlying disorder. Two visits were performed, respectively, at

<sup>20</sup> A postbiotic is defined as a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host (Salminen et al., 2021).

inclusion (V0) and at the end of the intervention period (V1). Data were collected by the paediatrician during the 2 visits and at home by the parents. Main outcomes were evaluation of the regurgitations with the Vandenplas scale; quality of life using the QUALIN questionnaire and analogical visual scale; crying duration; number and aspect of stools; and parents' satisfaction. The results analysis was carried on 692 infants, who consumed the thickened formula without major deviations to the protocol during the whole duration of the survey. No serious adverse events were reported. Because the study had an observational design, without a control group, the obtained information is of very limited value. Therefore, no conclusion can be drawn from this study on the safety of use of LBG in young infants.

The **LeopARd study** (final version of 5 June 2020; Documentation provided to EFSA n. 3) described in the document submitted to EFSA was a double-blind, randomised, controlled study, including infants with uncomplicated regurgitation. After inclusion, the infants were randomised to receive for 4 weeks either an anti-regurgitation (AR) test formula containing 0.4 g/100 mL LBG, 0.4 g/100 mL scGOS/lcFOS (ratio 9:1) and compounds derived from the Lactofidus<sup>TM</sup> fermentation process or a control AR formula. This control formula contained the same level of 0.4 g/100 mL LBG as the test product but contained a lower level of compounds derived from the Lactofidus<sup>TM</sup> fermentation process and no scGOS/lcFOS. All infants were exclusively formula fed for at least 7 days before randomisation. Infants received for at least 4 weeks one of two AR formulae. Infants were diagnosed with regurgitation, based upon the following adapted Rome IV criteria: (1) Regurgitation of  $\geq 2$  times per day for 1 or more weeks according to reported history; and (2) An indication for the use of AR formula according to the investigator at screening. Infants were  $> 3$  weeks and  $< 13$  weeks of age. The objective of the document submitted to EFSA was to provide available data on LBG, and therefore, the results of both study groups were combined, since both study groups received a formula with the same level of 0.4 g/100 mL LBG. Based on the interim analysis that was performed during the study, an independent external Data Monitoring Committee concluded that there were no indications for tolerance or safety concerns based on the available blinded interim data of the two study groups. In this document, the final results of the combined study groups are presented, and therefore the document refers to the study products, including the test and the control product. The visits were performed: the first at inclusion (baseline visit), the second at week 2 (V2) and the third at the end of the intervention period (V3). Data were collected by the paediatrician during the three visits and at home by the parents. Outcome parameters measured to assess the GI tolerance and safety were:

- 1) The Infant Gastrointestinal Symptom Questionnaire (IGSQ) that consists of 13 items divided in 5 clusters: stooling, spitting up/vomiting, crying, fussiness and flatulence;
- 2) The adapted Vandenplas score on regurgitation severity;
- 3) Parents scored the regurgitation of their infants daily in a diary during the 4 weeks of the study;
- 4) Stool frequency and stool consistency using the Amsterdam stool scale were respectively measured and assessed daily by the parents;
- 5) Adverse events and serious adverse events;
- 6) Growth. Anthropometric outcomes were assessed at every study visit and included weight, length and head circumference.

In total, 182 infants with regurgitation were included in the study, with a median age of 5.2 weeks at baseline. One infant did not take any study product and was, therefore, not included in the All-Subjects-Treated population (AST;  $n = 181$ ). During the 4-week study main phase, a total number of 32 subjects (out of the total number of 181 subjects in the AST population; 18%) terminated the study early. In total, there were 62 subjects (34%) with at least one reported (serious) adverse event (SAE). The most commonly reported AEs were GI disorders (in 18% of the subjects). In total, there were 11 reported SAEs in 10 subjects (5.5% of the total number of subjects), who all had recovered at the end of the study. Most of the SAEs were considered as not related or unlikely to be related to the use of study product. One SAE was assessed by the investigator as possibly related. This SAE was reported as 'gastroesophageal reflux disease without oesophagitis', which started 22 days after the subject started using the study product and lasted for 9 days. No SAE occurred in the study. The study population showed adequate growth during the study. Because the authors pooled the results from the two groups of infants randomised to receive two different AR formulae but containing the same amount of LBG (i.e., 0.4 g/100 mL), the study design is, indeed, observational and no more randomised. Therefore, the available information is of very limited value. No conclusion can be drawn from this document sent to EFSA on the safety of use of LBG in young infants.

The Panel was made aware by an IBO of an ongoing randomised controlled study (**SOLAR Study**) to assess the safety and tolerance of an infant formula with LBG in infants with regurgitation (Documentation provided to EFSA n. 4, 12–16). A total of 103 fully formula-fed healthy term infants, aged between 3 and 9 weeks at inclusion and diagnosed with uncomplicated regurgitation were included in the study. At the time of the drafting of this scientific opinion, only the preliminary results were available; therefore, the study cannot be considered for the safety assessment of E 410. The Panel further noted that there were methodological limitations in the study introducing high risk of bias.

Finally, the IBOs summarised in their submission a series of clinical studies from the literature addressing the use of LBG-thickened formula in the dietary management of uncomplicated GOR (Vanderplas et al., 1994, 2008, 2013; Georgieva, 2016; Dupont et al., 2016; Dupont and Vanderplas, 2020; Gatcheco et al., 2019). The Panel considers that no conclusions can be drawn from these clinical studies with respect to the assessment of LBG (E 410).

#### 3.5.2.4. Other studies

An IBO provided two case reports from the literature (Savino et al., 1999 and Jędrzejczyk et al., 2020; Documentation provided to EFSA n.3). Savino et al. (1999) was already assessed during the re-evaluation (see EFSA ANS Panel, 2017). Jędrzejczyk et al. (2020) described a case of food protein-induced enterocolitis syndrome (FPIES) triggered by LBG in an infant in an 11-week-old boy with chromosome 21 trisomy fed with cow's milk formula.

An *in vitro* study evaluating the effect of LBG on the mineral bioaccessibility was provided by the same IBO (Documentation provide to EFSA n. 3). In this study, the gastrointestinal digestion was simulated with a dialysis model to assess: (1) the *in vitro* mineral bioaccessibility of a LBG thickened anti-regurgitation-formula with increased calcium, iron and zinc levels compared to a non-thickened standard infant formula, (2) the effect of LBG addition to non-thickened standard infant formula on *in vitro* mineral bioaccessibility, and (3) the capability of 'current' anti-regurgitation (i.e. formula optimised to provide osmolality and mineral amounts that are appropriate for the needs of infants) to provide comparable amounts of bioaccessible mineral as non-thickened standard infant formula. The authors of the study concluded that the 'current' AR-formula provided a comparable amount of copper, and increased amounts of bioaccessible calcium, iron and zinc as non-thickened standard infant formula. The Panel considered that this *in vitro* study does not provide relevant information for the assessment of the safety of E 410 when used as a food additive.

#### 3.5.3. Post-marketing surveillance data

Post-marketing data were submitted from one IBO (Documentation provided to EFSA n.3) for two different companies.

Company 1 covered the period 1 January 2019 to 31 March 2020. In this period, 46 reports were sent to the IBO in total while more than 30 million units were placed on the market for a product described as Anti-regurgitation product with LBG, according to the information of the IBO. There were no serious adverse reactions among the 46 reports. Besides 1 case of allergic reaction, the reactions were unspecific (vomiting, diarrhoea, bloating) and a causal relationship to the ingestion of the product, described by the IBO, was not given.

Company 2 provided post-marketing data for the period 1 January 2017 to 31 October 2019. In this period, 769 reactions from 545 cases were reported to the IBO while more than 20 million units were placed on the market for a product described as LBG (E 410), according to the information of the IBO. There were no serious adverse reactions among the 768 reported reactions. The reactions were unspecific (diarrhoea, softer stool, flatulence) and a causal relationship to the ingestion of the product, described by the IBO, was not given.

#### 3.5.4. Benchmark dose modelling

The data from dose dependent changes in zinc and glucose levels in blood from the study from Ellemann-Laursen (2020; 2022; Documentation provided to EFSA n. 3,4,8) were used for benchmark dose modelling (see Section 3.5.2.2).

The selection of the BMR of 20% for **zinc** used in the BMD modelling is deduced from an epidemiological study performed in children with a mean age of 2.5 years (Cole et al., 2010). In this study, low Zn levels (< 10.7 µmol/L) were associated with a higher risk of anaemia (haemoglobin below 110 g/L) and serum zinc correlated with haemoglobin ( $r = 0.24$ ,  $p < 0.001$ ). In this study, a

20% decrease in Zn-level in humans (13.0  $\mu\text{mol/L}$  normal, to 10.4  $\mu\text{mol/L}$ ) is below the cut-off value for anaemia in this study and hence indicates an increased risk.

The selection of the BMR of 30% for **glucose** is based on textbook reference values for postprandial glucose where an increase to 1.4 g/L is considered normal and an increase to 1.8 g/L indicates diabetes (corresponding to an increase of 28.6%).<sup>21</sup> As the piglets were non-fasting, the postprandial glucose levels are considered to provide the most relevant information on the changes in this parameter.

The BMDL for zinc levels in blood resulted in 1,360 mg LBG/kg bw per day for males and 1,410 mg LBG/kg bw per day for females (see Appendix C). The BMDL for glucose levels in blood resulted in 1,450 mg LBG/kg bw per day for males and 1,530 mg LBG/kg bw per day for females (see Appendix D).

### 3.6. Discussion

The current assessment addresses the data gaps previously identified during the re-evaluation of locust bean gum (E 410) and the evaluation of the use in FC 13.1.5.1 for infants below 16 weeks of age.

Dietary exposure to LBG (E 410) for infants below 16 weeks of age from its use as a food additive was assessed based on (1) MPLs set out in the EU legislation (defined as the regulatory maximum level exposure assessment scenario) and (2) the reported use levels (defined as the refined exposure assessment scenario). Both scenarios are based on the recommended consumption levels from Scientific Committee Guidance (EFSA Scientific Committee, 2017) which recommends values of 200 and 260 mL formula/kg bw per day as conservative mean and high-level consumption values for 14- to 27-day-old infants.

For infants below 16 weeks of age consuming special infant formulae (FC 13.1.5.1), exposure to LBG (E 410) in the regulatory maximum level exposure assessment scenario was estimated to be 2,000 mg/kg bw per day for mean consumption while at the high-level consumption was estimated to be 2,600 mg/kg bw per day. Exposure estimates are the same in the refined scenario using the maximum use level reported by industry as this maximum equals the MPL. In the refined estimated exposure assessment scenario using the mean of the reported use levels from industry, exposure estimates for LBG (E 410) were of 869 mg/kg bw per day at the mean and 1,130 mg/kg bw per day at the high level of consumption.

Based on the assumption that carers of children with allergy or any other medical condition would be brand-loyal to an infant formula for special medical purposes (FC 13.1.5.1) that suits their medical condition, the Panel considers that the exposure assessment scenarios would in general result in reliable estimates of exposure.

For infants above 16 weeks of age and toddlers consuming special infant formulae (FCs 13.1.5.1 and 13.1.5.2), dietary exposure to LBG (E 410) was estimated in a FSMP consumers only scenario using the maximum reported use levels of 10,000 mg/kg for these FSMP. Mean exposure was estimated maximum to be 1,107 mg/kg bw per day for infants above 16 weeks of age and 653 mg/kg bw per day for toddlers; high-level exposure (p95) was estimated to be maximum 1,653 mg/kg bw per day for infants above 16 weeks of age and 1,225 mg/kg bw per day for toddlers.

In response to the call for data, analytical data on levels of four toxic elements (Pb, Hg, Cd and As) in commercial samples of LBG (E 410) (clarified and unclarified) were provided by IBOs. The Panel agreed that the lowest technologically achievable levels proposed by the IBOs for these toxic elements submitted for clarified LBG were consistent with the occurrence of data. However, the lowest technologically achievable levels, as well as the specifications proposed by the IBOs were much higher than most of the data reported for these four toxic elements in unclarified LBG.

The Panel performed the risk assessment that would result if Pb, Hg, Cd and As were present in E 410 at (i) the maximum current limit in the EU specification; (ii) the lowest technologically achievable levels proposed by the IBOs for unclarified LBG (E 410) (iii) the lowest technologically achievable levels proposed by the IBOs for clarified LBG (E 410).

The Panel considered the refined brand-loyal exposure scenario to calculate the exposure to the toxic elements from the use of E 410. For the general population, the highest mean and the highest 95th percentile in the refined brand-loyal exposure scenario were 237 and 435 mg/kg bw per day in toddlers (EFSA ANS Panel, 2017). For infants below 16 weeks of age, the refined exposure estimates

<sup>21</sup> <https://emedicine.medscape.com/article/2087913-overview>

based on the maximum and mean use levels of E 410 reported by industry (Table 7) were considered. The mean and 95th percentile exposure estimates were 2,000 and 2,600 mg/kg bw per day for the maximum use level and 839 and 1,130 mg/kg bw per day for the mean use level reported. The highest mean and 95th percentile exposure for the population above 16 weeks of age and toddlers (consumers only of FSMP) were calculated in this opinion as 1,107 and 1,653 mg/kg bw per day for infants above 16 weeks of age up to 1 year.

The Panel also calculated the impact of the potential level of the toxic elements lead and cadmium in the food additive (i.e. up to the specifications limit values) on the final product and compared that with the legal limits for these elements in the final formula for infants below 16 weeks of age set by Reg. (EC) No 1881/2006 (see Appendix E). Considering the results of these calculations and the fact that the food additive is not the only potential source of toxic elements in the infant formula the Panel emphasises the need to reduce the specification limit values for lead and cadmium in Regulation (EU) no 231/2012.

Consequently, the Panel concluded that the potential exposure to toxic elements resulting from the exposure to E 410 could be substantial (see Section 3.4.1).

The Panel noted that the maximum limits in the EU specifications for toxic elements should be established based on actual levels in the commercial food additive. Therefore, the Panel recommended that the maximum limits be lowered on the basis of the information provided by the IBO and on the considerations of the Panel (see Table 12). The Panel does not see the need to introduce separate specifications for toxic elements for unclarified and clarified LBG E 410.

In response to the call for data the IBOs provided information on current levels of residual proteins, TAMC, TYMC, *E. coli*, *Salmonella* spp. in E 410, which were within the specification of JECFA. One IBO also provided data on the absence of *Cronobacter* (*Enterobacter*) *sakazakii* in unclarified LBG (E 410) preparations.

Residual protein in commercial samples of unclarified LBG ranged up to 7% as reported by two IBOs, which is the current EU specification limit (Table 1). For clarified LBG, one IBO reported protein levels up to 0.5%. Although clarified LBG (E 410) contains lower levels of residual proteins than unclarified gum, the Panel does not see the need to introduce separate specifications for residual protein in unclarified and clarified LBG (E 410) because no indication for differences in adverse reactions are observed in clinical studies or newly submitted post-marketing surveillance data, where only one case of allergic reaction has been reported. Regarding the case reports only two studies reporting adverse events for two infants were provided (see Section 3.5.2.4).

The Panel noted that LBG (E 410) may be prone to microbiological contamination and therefore microbiological specifications should be set in the EU specifications. Although one IBO claimed that the process of clarification of LBG reduces the microbial load, and the limited data provided seem to support this, the Panel does not see the need to introduce separate microbiological specifications for unclarified and clarified LBG (E 410).

Based on the data on particle size distribution submitted by the IBOs and the criteria set in the relevant EFSA Scientific Committee Guidance (EFSA Scientific Committee, 2021), the Panel concluded that the presence of small particles, including nanoparticles, cannot be confirmed or excluded in the pristine food additive. The Panel noted that currently no standardised methods are available to measure the particle size distribution for the polysaccharide thickening and gelling agents used as food additives and that further research for method development is needed. The Panel noted, however, that polysaccharide thickening, and gelling agents used as food additives, to exert their technical function in general swell in liquid environments. This also applies to LBG (E 410). The FAF Panel considers that LBG will not be present in the GI-tract in the pristine form taking into account the capacity to absorb and swell in water, and the volume of fluid in the stomach and GI-tract.

Taking all these aspects into consideration, the Panel has made proposals for an update of the EU specifications for LBG (E 410) (see Table 12).

From the clinical studies provided only very limited information and none of them could be taken into account with respect to the safety of LBG administration because of the methodological limitations. The post-marketing data provided by the IBO did not show serious adverse events among over 50 million units sold except for one single case of allergic reaction.

No new data on absorption, distribution, metabolism and excretion and no new studies on acute, subchronic, genotoxic, developmental and reproductive toxicity were provided. From the data mentioned in the former ANS Panel opinion, it is to be noted that several studies were mentioned which suggested that the bioavailability of calcium, iron and zinc may be affected by thickening agents

(Bosscher et al., 2001; Gonzalez-Bermudez et al., 2014) whereas one human study did not support this suggestion using low doses of 200–300 mg/kg per day (Behall et al., 1987).

A new study in neonatal piglets which were given LBG (E 410) was provided by one IBO; the amount consumed being calculated as on average 0, 1,050, 1,500 or 2,400 g/kg bw per day for the control formula and formulae with 3.5, 5.0 or 8.0 g LBG/L. Changes considered related to the treatment with LBG included increased weight of segments of large and small intestines. As no related clinical signs, macroscopic and microscopic changes were observed, these changes are considered of no toxicological relevance. The changes in red blood cell parameters might be related to the observed decrease in zinc levels. Decreases in blood zinc concentration were considered of toxicological importance since zinc deficiency is known to correlate with a number of biological effects. The increase in blood glucose is considered of toxicological importance although the toxicological relevance with respect to long term adverse apical effects is less clear for the infant age and for the short life span of exposure towards LBG than in adults where micro- macrovascular changes in the retina, the kidney and blood vessels leads to blindness, renal insufficiency, and cardiovascular diseases (Cheung et al., 2010; Gärtner and Eigentler, 2008; Lu et al., 2020).

Zinc is an essential element for the functioning of the human body as it is involved in the activation of more than 300 enzymes regulating important biological processes. In addition, zinc stabilises the tertiary structure of a variety of proteins, in particular Zn finger proteins, which have an important role in growth and development (Hussain et al., 2022). In an epidemiological study of Cole et al. (2010), low Zn levels ( $< 10.7 \mu\text{mol/L}$ ) were associated with a higher risk of anaemia (haemoglobin below 110 g/L). In this study, serum zinc correlated with haemoglobin ( $r = 0.24$ ,  $p < 0.001$ ). The selection of the BMR of 20% used in the BMD modelling (see below) is deduced from this study as a 20% decrease in Zn-level in humans (13.0  $\mu\text{mol/L}$  normal, to 10.4  $\mu\text{mol/L}$ ) is below the cut off value for anaemia in this study (Cole et al., 2010).

Concerning the underlying mechanism for increased non-fasting blood glucose, the Panel considered that the decreased zinc blood concentration may be responsible for the observed increase in blood glucose. Zinc is involved in the synthesis, processing and secretion of insulin in pancreatic  $\beta$ -cells (Cole et al., 2010; Poudel et al., 2017; Hussain et al., 2022). Zinc takes part in the inhibition of glucagon secretion in response to high glucose concentrations. Zinc regulates expression and activity of Insulin Degrading Enzyme (atypical zinc-metalloproteinase). Thus, zinc is involved in many steps in the blood sugar regulation. Hence, a low zinc blood level may lead to low insulin and a high glucagon which results in increased blood glucose levels. Insofar, elevated blood glucose may be secondary to decreased zinc blood levels.

The mechanism of the decreased zinc blood levels is binding of zinc to the LBG with a major influence on the availability of zinc as demonstrated *in vitro* by Bosscher et al. (2001). Gonzalez-Bermudez et al. (2014) also showed an influence on the availability of zinc by LBG in an *in vitro* experiment.

The data from dose dependent changes in zinc and glucose levels in blood from the study on neonatal piglets (Ellemann-Laursen, 2020; 2022) were used for BMD modelling. As mentioned above, the selection of the BMR of 20% for zinc used in the BMD modelling is deduced from an epidemiological study (Cole et al., 2010). The selection of the BMR of 30% for glucose is derived from textbook reference values for postprandial glucose where an increase from 1.4 g/L is normal and an increase to 1.8 g/L indicates diabetes (corresponding to an increase of 28.6%). For zinc, the resulting BMDL amounted to 1,360 mg LBG/kg bw per day for males and 1,410 mg LBG/kg bw per day for females. For glucose the resulting BMDL amounted to 1,450 mg LBG/kg bw per day for males and 1,530 mg LBG/kg bw per day for females.

Considering the above, a RP of 1,400 mg/kg bw per day was identified.

It could also be considered to do the dose–response modelling on the basis of the concentrations in formula as given in the piglet study report. This might better reflect the interaction of LBG with zinc bioavailability.

Following the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010 (EFSA, 2014), the former ANS Panel concluded that there is no need for a numerical ADI for LBG (E 410). The FAF Panel considered that the MoE approach would be the most appropriate mean for assessing the safety of LBG (E 410) when used as a food additive in the infant and young children population.

For the assessment of the MoE, the default uncertainty factor of 100 takes into consideration the interspecies differences as well as the intraspecies variability in toxicokinetics and toxicodynamics. However, as the mechanism leading to low zinc levels in blood is a chemical or physicochemical interaction in the gastrointestinal tract with LBG, interspecies differences and also intraspecies

variability in toxicodynamics are unlikely. Because LBG is not systemically available, and the chemical/physicochemical interaction takes place in the GI tract also no toxicokinetic interspecies difference or intraspecies variability has to be considered. Hence, the Panel concluded that, using the RP of 1,400 mg/kg bw per day based on the BMDL for adverse effects on zinc levels, a MoE above 1 would not raise a safety concern.

For infants below 16 weeks of age consuming FSMPs (FC 13.1.5.1), the MoE would be below 1 for the regulatory maximum level exposure scenario as well as for the maximum use level reported by industry; using mean use levels reported by the IBO, the MoE would be 1.6 for mean and 1.2 for high-level consumption (95th percentile), respectively.

For infants above 16 weeks of age up to 1-year consumers only of FSMPs, see reported use levels in Section 3.4.1, with a RP of 1,400 mg/kg bw per day, the MoE would be above 1 for the mean consumption and below 1 for the high-level consumption.

For toddlers (young children) consumers only of FSMPs the exposure estimates calculated in the current opinion were used. The same RP as for infants was used as the same effects related to reduced zinc absorption are expected and it was assumed that toddlers would have no higher sensitivity for decreased zinc levels. For this scenario, the MoEs would be above 1 for both the mean and high-level consumption.

Had the dose-response modelling been based on concentrations in formula the resulting conclusions would have been the same.

## 4. Conclusions

The Panel concluded that the technical data provided by the IBO support an amendment of the specifications for LBG (E 410) laid down in Commission Regulation (EU) No 231/2012, as presented by the recommendations made in Table 12. The Panel agreed that there is no need to introduce separate specifications for clarified and unclarified LBG.

The Panel identified a RP of 1,400 mg/kg bw per day based on reduced blood zinc levels in a piglet study. It applied the MoE for the safety assessment of LBG (E 410) when used as a food additive in FC 13.1.5.1 and 13.1.5.2. The Panel concluded that a MoE above 1 would not raise a safety concern.

With respect to the safety of the use of LBG (E 410) for infants below 16 weeks of age consuming FSMPs (FC 13.1.5.1), a MoE above 1 was obtained when the average typical use levels reported by the IBO were considered (mean and 95th percentile). However, when exposure estimates are based on the maximum permitted level or on the maximum use level reported by the IBO (mean and 95th percentile) MoEs below 1 were obtained.

With respect to the safety of use for infants above 16 weeks of age until 1-year consumers only of FSMPs (FC 13.1.5.1 and FC 13.1.5.2), a MoE above 1 was obtained for the mean consumption but not for the consumption at the 95th percentile.

With respect to the safety in use for toddlers (young children) consumers only of FSMPs (FC 13.1.5.2), a MoE above 1 was given for all exposure levels.

## 5. Documentation as provided to EFSA

- 1) Association of Producers of Carob Bean Gum (INEC), 2020. Submission of data in response to the call for technical and toxicological data on locust bean gum (E 410) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on 29 October 2020.
- 2) Association of Producers of Carob Bean Gum (INEC), 2021. Additional data and clarifications submitted following the call for technical and toxicological data on locust bean gum (E 410) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on 30 July 2021.
- 3) Specialised Nutrition Europe (SNE), 2020. Submission of data in response to the call for technical and toxicological data on locust bean gum (E 410) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on 29 October 2020.
- 4) Specialised Nutrition Europe (SNE), 2021. Additional data and clarifications submitted following the call for technical and toxicological data on locust bean gum (E 410) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on 3 September 2021.

- 5) CP-Kelco, 2020. Submission of data in response to the call for technical and toxicological data on locust bean gum (E 410) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on 8 September 2020.
- 6) CP-Kelco, 2021. Additional data and clarifications submitted following the call for technical and toxicological data on locust bean gum (E 410) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on 22 July 2021.
- 7) Association of Producers of Carob Bean Gum (INEC), 2022. Additional data and clarifications submitted following the call for technical and toxicological data on locust bean gum (E 410) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on 22 September 2022.
- 8) Specialised Nutrition Europe (SNE), 2022. Additional data and clarifications submitted following the call for technical and toxicological data on locust bean gum (E 410) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on 21 June 2022.
- 9) Specialised Nutrition Europe (SNE), 2022. Additional data and clarifications submitted following the call for technical and toxicological data on locust bean gum (E 410) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on 28 October 2022.
- 10) CP-Kelco, 2022. Additional data and clarifications submitted following the call for technical and toxicological data on locust bean gum (E 410) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on 23 September 2022.
- 11) CP-Kelco, 2022. Additional data and clarifications submitted following the call for technical and toxicological data on locust bean gum (E 410) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on 25 October 2022.
- 12) Specialised Nutrition Europe (SNE), 2019. Update on the ongoing randomised, controlled study to evaluate the safety and tolerance of an infant formula with locust bean gum in infants with regurgitation (SOLAR study). Submitted on 21 October 2019.
- 13) Specialised Nutrition Europe (SNE), 2019. Update and clarifications on the ongoing randomised, controlled study to evaluate the safety and tolerance of an infant formula with locust bean gum in infants with regurgitation (SOLAR study). Submitted on 18 December 2019.
- 14) Specialised Nutrition Europe (SNE), 2019. Update and clarifications on the ongoing randomised, controlled study to evaluate the safety and tolerance of an infant formula with locust bean gum in infants with regurgitation (SOLAR study). Submitted on 18 December 2019.
- 15) Specialised Nutrition Europe (SNE), 2020. Update and clarifications on the ongoing randomised, controlled study to evaluate the safety and tolerance of an infant formula with locust bean gum in infants with regurgitation (SOLAR study). Submitted on 14 February 2020.
- 16) Specialised Nutrition Europe (SNE), 2022. Update and clarifications on the ongoing randomised, controlled study to evaluate the safety and tolerance of an infant formula with locust bean gum in infants with regurgitation (SOLAR study). Submitted on 8 September 2022.
- 17) Association of Producers of Carob Bean Gum (INEC), 2022. Additional data and clarifications submitted following the call for technical and toxicological data on locust bean gum (E 410) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on 31 October 2022.

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## Abbreviations

(S)AE	(serious) adverse event
AAS	atomic absorption spectroscopy
ADI	acceptable daily intake
ANS Panel EFSA	Panel on Food Additives and Nutrient Sources added to Food
AST	All-Subjects-Treated population
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDU	benchmark dose upper confidence limit
bw	body weight
CAS	Chemical Abstract Service
CFU	colony forming unit
FAF Panel	Panel on Food Additives and Flavourings
FAO/WHO	Food and Drug Organisation/World Health Organisation
FC	food category
FOS	fructo-oligosaccharides
FSMP	food for special medical purposes
GI	gastrointestinal
GOR	gastro-oesophageal reflux
GOS	galacto-oligosaccharides
HBGV	health-based guidance value
IBO	interested business operator
ICP-MS	inductively coupled plasma mass spectrometry
IGSQ	Infant Gastrointestinal Symptom Questionnaire

IQ	intelligence quotient
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LBG	locust bean gum
LD	laser diffraction
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOQ	limit of quantification
Mintel GNPD	Mintel's Global New Products Database
MOE	margin of exposure
MOS	margin of safety
MPL	maximum permitted level
NMT	not more than
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
PAG	polyacrylamide gel
PND	postnatal day
RP	reference point
SC	Scientific Committee of EFSA
SCF	Scientific Committee on Food
TAMC	total aerobic microbial count
TWI	tolerable weekly intake
TYMC	total combined yeast and mould count
WG	Working Group

## Appendix A – Data requested in the call for data (Call for technical and toxicological data on locust bean gum (E 410) for uses as a food additive in foods for all population groups including infants below 16 weeks of age<sup>22</sup>

Kind of data	Data requested in the call for data	Responses from interested business operators	Comment
<b>A. Information regarding the follow-up of the conclusions and the recommendations of the EFSA ANS Panel opinion on the safety of locust bean gum (E 410) as a food additive</b>			
<b>1. Technical data</b>	<ul style="list-style-type: none"> <li>analytical data on current levels of lead, mercury, cadmium and arsenic in commercial samples of the food additive;</li> <li>the lowest technologically achievable level for lead, mercury, cadmium, and arsenic in order to adequately define their maximum limits in the specifications;</li> <li>current levels of residual proteins in clarified and unclarified preparations;</li> <li>the possibility to use clarified locust bean gum to cover all technological needs of the food additive E 410;</li> <li>the lowest technologically achievable level for residual proteins in clarified and unclarified preparations in order to adequately define their maximum limits in the specifications in view of case reports on hypersensitivity reactions associated with locust bean gum.</li> </ul> <p>In addition, a proposal for separate specifications for clarified and unclarified locust bean gum is requested.</p>	Data submitted	Assessed, no further follow up
<b>2. Toxicological data</b>	According to the conclusions and recommendations in the Scientific opinion on the re-evaluation of locust bean gum (E 410) as a food additive by the EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food) published in 2017, the generation of additional data to assess the potential health effects of locust bean gum (E 410) when used as a food additive in 'dietary foods for infants for special medical purposes and special formulae for infants' (Food category 13.1.5.1) and in 'dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC' (Food category 13.1.5.2) was recommended. These requirements could be addressed as outlined in Section B.2.	Data submitted	Assessed, no further follow up
<b>3. Literature searches</b>	Literature searches should be conducted relevant for the safety evaluation of locust bean gum (E 410) for all uses in foods for all population groups from 12/10/2016 up to the date of the data submission, as described in the Guidance for submission for food additive evaluations (see its Section 5.3).	Data submitted	Assessed, no further follow up

<sup>22</sup> Available from: <https://www.efsa.europa.eu/en/consultations/call/181010-4> and responses from interested business operators.

Kind of data	Data requested in the call for data	Responses from interested business operators	Comment
<b>B. Information required for the risk assessment of locust bean gum (E 410) as a food additive for use in foods for infants below 16 weeks of age</b>			
<b>1. Technical data</b>	<p>For the uses of locust bean gum (E 410) as a food additive in foods for infants below 16 weeks EFSA seeks information on:</p> <ul style="list-style-type: none"> <li>the usage levels of locust bean gum (E 410), alone or in combination with other thickening agents (indication of food additive name and level of use) in the special formulae for infants below 16 weeks of age under special medical conditions (FC 13.1.5.1);</li> <li>the fate and the reaction products of locust bean gum (E 410) in special formulae for infants below 16 weeks of age under special medical conditions (FC 13.1.5.1);</li> <li>particular specification requirements for identity and the purity of locust bean gum (E 410) (e.g. with respect to levels of protein residues; use of clarified locust bean gum; content of toxic elements, propan-2-ol/ethanol) for special formulae for infants below 16 weeks of age under special medical conditions; analytical data on impurities in the final special formulae for infants below 16 weeks of age need to be provided when no legal limit has been established;</li> </ul> <p>In addition, data should be provided demonstrating the absence of <i>Cronobacter (Enterobacter) sakazakii</i> in the food additive.</p>	Data submitted	Assessed, no further follow up
<b>2. Toxicological data</b>	<ul style="list-style-type: none"> <li>a repeated dose study with direct oral administration of locust bean gum (E 410) to neonatal animals which includes gross and histopathological examination of the gastrointestinal tract, influence on the microbiota and a possible modification of the bioavailability of nutrients (vitamins and minerals, such as calcium, iron and zinc), that are normally contained in food for infants. The study shall be performed in piglets unless justification for the relevance of a study in another species is given;</li> <li>clinical data focusing on gastrointestinal effects to assess the safety of locust bean gum (E 410) when used in dietary foods for special medical purposes in infants below 16 weeks of age (FC 13.1.5.1);</li> <li>post-marketing surveillance reports on undesired and adverse reactions (including e.g. flatulences, gastrointestinal discomfort, changes of stool-frequencies and -consistency, diarrhoea and allergic reactions), indicating the ages and other relevant data of the exposed infants and young children and the use level of locust bean gum (E 410) in the marketed products;</li> </ul>	Data submitted	Assessed, no further follow up

Kind of data	Data requested in the call for data	Responses from interested business operators	Comment
	<ul style="list-style-type: none"> <li>published and unpublished case reports (e.g. available nutrivigilance data) on undesired and adverse effects, including e.g. flatulences, gastrointestinal discomfort, changes of stool-frequencies and -consistency, diarrhoea and allergic reactions, associated with the oral administration of locust bean gum in any form to infants and young children.</li> </ul>		
<b>3. Literature searches</b>	Literature searches should be conducted relevant for the safety evaluation locust bean gum (E 410) when used in foods for infants below 16 weeks of age up to the date of the data submission, as described in the Guidance for submission for food additive evaluations (Section 5.3).	Data submitted	Assessed, no further follow up

## Appendix B – Risk of bias/Internal validity for Experimental Animal Studies (modified from to NTP 2015; 2019)

**Study:** Locust Bean Gum Piglet Adapted Formula Study in Juvenile Minipigs (Test Facility Study No. 78627, S. Ellemann-Laursen, 2020; documentation provided to EFSA n. 3,4,8).

### Decision rules

The ratings of the key and non-key questions (+++, +, -, --) will be integrated to classify the studies in tiers from 1 to 3 corresponding to decreasing levels of internal validity.

#### Tier 1:

All the key questions are scored +/++  
AND  
No more than one non-key question is scored –  
AND  
No non-key question is scored –

#### Tier 2:

All the other combinations not falling under tier 1 or 3

#### Tier 3:

Any question is scored --  
OR  
More than one key question is scored –

### Overall outcome: Tier 1

Number	Question	Domain of bias	Rating (+++, +, -, --)	Reviewer 1	Reviewer 2
1*	<b>Was administered dose or exposure level adequately randomised? (Please apply the question also on F1 and F2 generation) Key question</b>	Selection	<p>++ if the method is described and it is adequate</p> <p>+ if the authors only indicate that randomisation was done but do not describe the method</p> <p>– no mentioning of randomisation</p> <p>-- direct evidence of no randomisation</p>	++ The piglets were allocated in order to distribute littermates evenly between groups and in order to reduce inter-group mean body weight differences	++ Mean body weight differences and data available from pre-treatment observations were used for the allocation.
2	<b>Was allocation to study groups adequately concealed?</b>	Selection	<p>++ properly concealed and described how concealment was performed</p> <p>+ mentioning that concealment was performed; + is also appropriate if non-concealment does not influence the outcome</p> <p>– if non-concealment does influence the outcome (measurements with a subjective part (e.g. preparation of fat pads, observation of behaviour))</p>	++ Properly concealed and described how concealment was performed	+ Piglets identified by number and colour code

Number	Question	Domain of bias	Rating (++, +, -, --)	Reviewer 1	Reviewer 2
			-- if non-concealment does influence the outcome to a very important part (subjective measurements)		
3*	<b>Were experimental conditions identical across study groups?</b> Key question	Performance	<div style="background-color: green; padding: 2px;">++ experimental conditions described and identical across study groups (feeding, water supply, bedding, day/night cycle; temperature; humidity)</div> <div style="background-color: #c6e0b4; padding: 2px;">+ incomplete description of experimental conditions; + is also appropriate if lack of information does not influence the outcome</div> <div style="background-color: yellow; padding: 2px;">- if lack of information does influence the outcome</div> <div style="background-color: red; padding: 2px;">-- if factors clearly indicate that treatment conditions were different does influence the outcome to a very important part</div>	++ Experimental conditions are described as identical.	++ Experimental conditions are described as identical.
4*	<b>Was the research personnel blinded to the study group?</b> Key question	Performance	<div style="background-color: green; padding: 2px;">++ if there is direct evidence that the research personnel did not know what group animals were allocated to, and it is unlikely that they could have broken the blinding of allocation</div> <div style="background-color: #c6e0b4; padding: 2px;">+ if not reported and lack of adequate allocation concealment could not appreciably affect the handling/outcome of measurements of different study groups (e.g. methods used which do not have a subjective component)</div> <div style="background-color: yellow; padding: 2px;">- if not reported and lack of adequate allocation concealment could appreciably affect the handling/measurement of different study groups (e.g. methods used which have a subjective component)</div>	+ Not reported and lack of adequate allocation concealment would not appreciably affect the allocation of animals to different study groups (e.g. methods used which do not have a subjective component)	+ Not reported but without impact on results

Number	Question	Domain of bias	Rating (++, +, -, --)	Reviewer 1	Reviewer 2
			-- if there is direct evidence that it was possible for the research personnel to know what group animals were allocated to, or it is likely that they could have broken the blinding of allocation		
5	<b>Were outcome data complete without attrition or exclusion from analysis?</b>	Attrition/exclusion	++ There is direct evidence that loss of animals was adequately addressed and reasons were documented when animals were removed from a study. OR Missing data have been imputed using appropriate methods (ensuring that characteristics of animals are not significantly different from animals retained in the analysis).  + There is indirect evidence that loss of animals was adequately addressed, and reasons were documented when animals were removed from a study. OR It is deemed that the proportion lost would not appreciably bias results. This would include reports of no statistical differences in characteristics of animals removed from the study from those remaining in the study. OR There is insufficient information provided about loss of animals (record 'NR' as basis for answer) but it is considered that this does not have an impact on the validity of the study.	++ Direct evidence that loss of animals was adequately addressed, and reasons were documented when animals were removed from a study.	++ One animal was reported to be euthanised prematurely, otherwise no loss of animals and no missing data.
			– There is indirect evidence that loss of animals was unacceptably large and not adequately addressed (e.g. if		

Number	Question	Domain of bias	Rating (++, +, -, --)	Reviewer 1	Reviewer 2
			<p>unexplained loss is equal or more than 25%). OR There is insufficient information provided about loss of animals (record 'NR' as basis for answer) and it is suspected that this would have an impact on the validity of the study.</p> <p>Note: Unexplained inconsistencies between materials and methods and results sections (e.g. inconsistencies in the numbers of animals in different groups) could be an example of indirect evidence.</p> <p>-- There is direct evidence that loss of animals was unacceptably large and not adequately addressed.</p>		
6*	<p><b>Can we be confident in the exposure characterisation?</b> <b>Key question</b></p>	Detection	<p>++ There is direct evidence that the substance was sufficiently described and consistently administered (e.g. with the same method and timeframe) across treatment groups.</p> <p>+ There is indirect evidence that the substance was sufficiently described and consistently administered (i.e. with the same method and time-frame) across treatment groups. OR There is insufficient information provided about description and administration of the substance (record 'NR' as basis for answer) but it is considered that this does not have an impact on the validity of the study.</p> <p>- There is indirect evidence that the substance was not sufficiently described and was not consistently administered (e.g. with the same method and</p>	<p>++ There is direct evidence that the substance was sufficiently described and consistently administered (e.g. with the same method and timeframe) across treatment groups.</p>	<p>++ Batch number and Certificate of analysis were provided. Treatment via formula was clearly reported.</p>

Number	Question	Domain of bias	Rating (++, +, -, --)	Reviewer 1	Reviewer 2
			timeframes) across groups. OR There is insufficient information provided about description and administration of the substance (record 'NR' as basis for answer) and it is suspected that this has an impact on the validity of the study.  -- There is direct evidence that the substance was not sufficiently described and/or was not consistently administered (e.g. with the same method and timeframes) across groups.		
7*	<b>Can we be confident in the outcome assessment?</b> <b>Key question</b>	Detection			
		<b>Element 1</b> Was the outcome assessed at the same length of time (i.e. day and/or time of day) after initial exposure in all study groups? (remember to take into consideration the endpoints assignments) <b>Element 2</b> Was a reliable and sensitive animal model used for investigating the test compound and selected endpoints? <b>Element 3</b> Was the number of animals per dose group appropriate?	++ There is direct evidence  + It is deemed that deviation would not appreciably bias results. OR There is insufficient information provided but it is considered that this does not have an impact on the validity of the study.  - There is insufficient information provided (record 'NR' as basis for answer) and it is suspected that this has an impact on the validity of the study.  -- There is direct evidence for a deviation.	++ All elements: ++, Animal number was low but acceptable	Elements 1 – 7: ++ # 3: Number of animals was low but acceptable # 5: Treatment started only at PND 7/8 (for 21 days) which is considered acceptable.

Number	Question	Domain of bias	Rating (++, +, -, --)	Reviewer 1	Reviewer 2
		<p><b>Element 4</b> Was the number of animals per sex in each cage appropriate for the study type and animal model?</p> <p><b>Element 5</b> Was the timing and duration of administration of the test compound appropriate?</p> <p><b>Element 6</b> Were reliable and sensitive test methods used for investigating the selected endpoints?</p> <p><b>Element 7</b> Were the measurements collected at suitable time points in order to generate sensitive, valid and reliable data?</p>			
8	<b>Were all outcomes measured according to the methodology section reported?</b>	Selective reporting	<p>++ There is direct evidence that all of the study's measured outcomes (apical and intermediate) outlined in the protocol, methods, abstract, and/or introduction that are relevant for the evaluation have been reported. This would include outcomes reported with sufficient detail to be included in meta-analysis or fully tabulated during data extraction and analyses had been planned in advance.</p> <p>+ There is indirect evidence that all of the study's measured outcomes (apical and intermediate) outlined in</p>	<p>++ There is direct evidence that all the study's measured outcomes (apical and intermediate) outlined in the protocol, methods, abstract, and/or introduction that are relevant for the evaluation have been reported.</p>	<p>++ Measurements done according to Materials &amp; Methods are considered appropriate.</p>

Number	Question	Domain of bias	Rating (++, +, -, --)	Reviewer 1	Reviewer 2
			<p>the protocol, methods, abstract, and/or introduction that are relevant for the evaluation have been reported. This would include outcomes reported with insufficient detail such as only reporting that results were statistically significant (or not). OR Analyses that had not been planned in advance (i.e. retrospective unplanned subgroup analyses) are clearly indicated as such and it is deemed that the unplanned analyses were appropriate and selective reporting would not appreciably bias results (e.g. appropriate analyses of an unexpected effect). OR There is insufficient information provided about selective outcome reporting (record 'NR' as basis for answer) but it is considered that this does not have an impact on the validity of the study.</p>		
			<p>– There is indirect evidence that all of the study's measured outcomes (apical and intermediate) outlined in the protocol, methods, abstract, and/or introduction that are relevant for the evaluation have not been reported. OR There is indirect evidence that unplanned analyses were included that may appreciably bias results. OR There is insufficient information provided about selective outcome reporting (record 'NR' as basis for answer) and it is suspected that this has an impact on the validity</p>		

Number	Question	Domain of bias	Rating (++, +, -, --)	Reviewer 1	Reviewer 2
			of the study.  Note: Unexplained inconsistencies between materials and methods and results/abstract or summary sections (e.g. inconsistencies in the numbers of animals in different groups) could be an example of indirect evidence.		
			-- There is direct evidence that not all of the study's measured outcomes (apical and intermediate) outlined in the protocol, methods, abstract, and/or introduction that are relevant for the evaluation have not been reported. In addition to not reporting outcomes, this would include reporting outcomes based on composite score without individual outcome components or outcomes reported using measurements, analysis methods or subsets of the data that were not pre-specified or reporting outcomes not pre-specified, or that unplanned analyses were included that would appreciably bias results.		
9	Were statistical methods appropriate?	Other sources of bias	++ There is direct evidence that the statistical methods seem appropriate and were clearly reported (adequate treatment of multiple testing)  + Statistical methods were not clearly reported but it may be inferred from other information that they were appropriate. OR There is insufficient information provided about statistical methods (record 'NR' as basis for	++ There is direct evidence that the statistical methods seem appropriate and were clearly reported	++ The statistical methods seem appropriate and were clearly reported

Number	Question	Domain of bias	Rating (++, +, -, --)	Reviewer 1	Reviewer 2
			<p>answer) but it is considered that this does not have an impact on the validity of the study.</p> <p>– Statistical methods were not clearly reported but it may be inferred from other information that they were not appropriate. OR There is insufficient information provided about statistical methods (record 'NR' as basis for answer) and it is suspected that this has an impact on the validity of the study.</p> <p>— There is direct evidence that the statistical methods applied were inappropriate.</p>		

## Appendix C – Benchmark Dose Modelling from the study in juvenile minipigs Zinc levels in blood

### Data Description

The endpoint to be analysed is: Zinc levels in blood (mean).

The data used in the analysis are summarised in Table C.1.

The analysis considered the study 'Locust Bean Gum Piglet Adapted Formula Study in Juvenile Minipig' (Test Facility Study No. 78627, S. Ellemann-Laursen, 2020 and the final amended report No 1 of this study, 2022; documentation provided to EFSA n. 3,4,8).

**Table C.1:** Data used for analysis

Correct.Dose (average g/kg bw per day)	Znmean (µmol/L)	ZnSD	ZnN	sex <sup>(a)</sup>
0.00	27.67	2.25	6	1
1.05	24.83	3.54	6	1
1.50	23.33	3.08	6	1
2.40	20.00	2.97	6	1
0.00	27.17	1.94	6	2
1.05	25.50	1.87	6	2
1.50	22.33	2.34	6	2
2.40	20.83	2.04	6	2

(a): 1 = males; 2 = females.

### Selection of the BMR

The BMR (benchmark response) used is a 20% change in mean response compared to the controls. The selection of the BMR of 20% for zinc used in the BMD modelling is deduced from an epidemiological study, performed in children with a mean age of 2.5 years (Cole et al., 2010). In this study, low zinc levels (< 10.7 µmol/L) were associated with a higher risk of anaemia (haemoglobin below 110 g/L) and serum zinc correlated with haemoglobin ( $r = 0.24$ ,  $p < 0.001$ ). In this study, a 20% decrease in Zn-level in humans (13.0 µmol/L normal, to 10.4 µmol/L) is below the cut off value for anaemia in this study and hence indicates an increased risk.

The BMD (benchmark dose) is the dose corresponding with the BMR of interest.

A 90% confidence interval around the BMD will be estimated, the lower bound is reported by BMDL and the upper bound by BMDU.

### Software Used

Results are obtained using the EFSA web-tool for BMD analysis, which uses the R-package PROAST, version 70.0, for the underlying calculations.

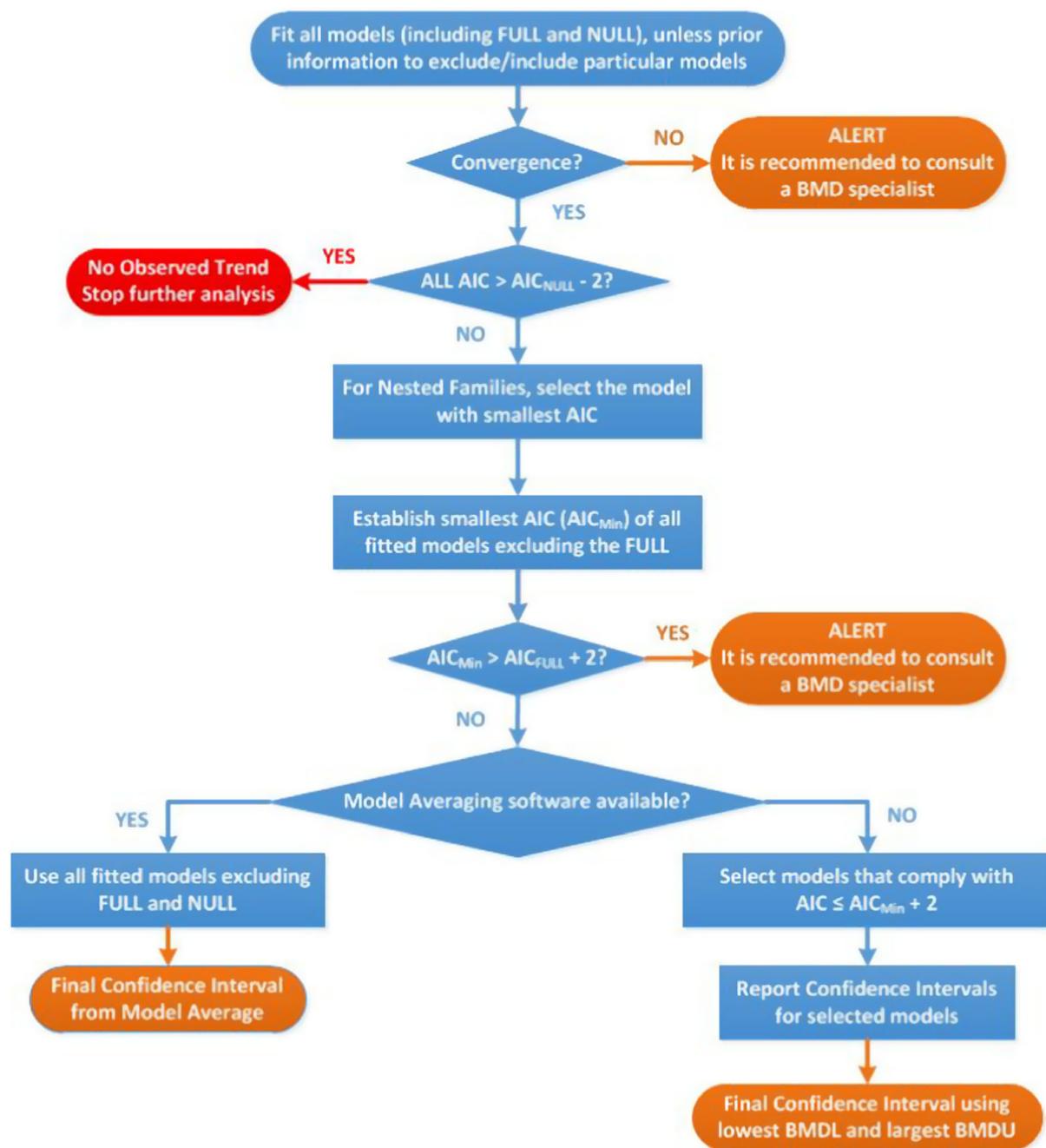
### Dose-response models

Default set of fitted models:

Model	Number of parameters	Formula
Null	1	$y = a$
Full	no. of groups	$y = \text{groupmean}$
Exp model 3	3	$y = a \cdot \exp(bx^d)$
Exp model 4	4	$y = a \cdot (c - (c-1)\exp(-bx^d))$
Hill model 3	3	$y = a \cdot \left(1 - \frac{x^d}{b^d + x^d}\right)$
Hill model 4	4	$y = a \cdot \left(1 - \frac{(c-1) \cdot x^d}{b^d + x^d}\right)$
Inverse Exponential	4	$y = a \cdot (1 + (c-1)\exp(-bx^{-d}))$
Log-Normal Family	4	$y = a \cdot (1 + (c-1)\Phi(\ln b + \ln x))$

As a covariate is included in the analysis, these models will also be fitted assuming that some of the parameters [background response parameter (a), potency parameter (BMD) and/or variance (var)] depend on the subgroup defined by the covariate. Therefore, the number of parameters in each model might be larger than indicated in the table above.

**Procedure for selection of BMDL**



Flowchart for selection of BMDL

**Results**

Response variable: Znmean

**Fitted Models**

Model	converged	loglik	npar	AIC
full model	yes	42.24	9	-66.48
full-v	yes	43.91	10	-67.82
null model-v	yes	23.16	3	-40.32
null model-a-v	yes	23.17	4	-38.34

Model	converged	loglik	npar	AIC
Expon. m3-v	yes	42.25	5	-74.50
Expon. m3-av	yes	42.27	6	-72.54
Expon. m3-bv	yes	42.38	6	-72.76
Expon. m3-abv	yes	42.44	7	-70.88
Expon. m5-v	yes	43.13	6	-74.26
Expon. m5-av	yes	43.15	7	-72.30
Expon. m5-bv	yes	43.16	7	-72.32
Expon. m5-abv	yes	43.31	8	-70.62
Hill m3-v	yes	42.25	5	-74.50
Hill m3-av	yes	42.27	6	-72.54
Hill m3-bv	yes	42.38	6	-72.76
Hill m3-abv	yes	42.44	7	-70.88
Hill m5-v	yes	43.04	6	-74.08
Hill m5-av	yes	43.06	7	-72.12
Hill m5-bv	yes	43.05	7	-72.10
Hill m5-abv	yes	43.06	8	-70.12
Inv.Expon. m3-v	yes	42.38	5	-74.76
Inv.Expon. m3-av	yes	42.40	6	-72.80
Inv.Expon. m3-bv	yes	42.49	6	-72.98
Inv.Expon. m3-abv	yes	42.55	7	-71.10
Inv.Expon. m5-v	yes	42.52	6	-73.04
Inv.Expon. m5-av	yes	42.54	7	-71.08
Inv.Expon. m5-bv	yes	42.62	7	-71.24
Inv.Expon. m5-abv	yes	42.67	8	-69.34
LN m3-v	yes	42.32	5	-74.64
LN m3-av	yes	42.34	6	-72.68
LN m3-bv	yes	42.44	6	-72.88
LN m3-abv	yes	42.51	7	-71.02
LN m5-v	yes	42.57	6	-73.14
LN m5-av	yes	42.59	7	-71.18
LN m5-bv	yes	42.65	7	-71.30
LN m5-abv	yes	42.70	8	-69.40

## Estimated Model Parameters

### EXP

estimate for var-1: 0.01387  
 estimate for var-2: 0.00731  
 estimate for a-: 27.36  
 estimate for CED-: 1.889  
 estimate for d-: 1.237

### HILL

estimate for var-1: 0.01387  
 estimate for var-2: 0.007309  
 estimate for a-: 27.36  
 estimate for CED-: 1.888  
 estimate for d-: 1.241

### INVEXP

estimate for var-1: 0.01387  
 estimate for var-2: 0.00723

estimate for a-: 27.34  
 estimate for CED-: 1.871  
 estimate for d-: 0.2333

**LOGN**

estimate for var-1: 0.01387  
 estimate for var-2: 0.007265  
 estimate for a-: 27.35  
 estimate for CED-: 1.879  
 estimate for d-: 0.4271

**Weights for Model Averaging**

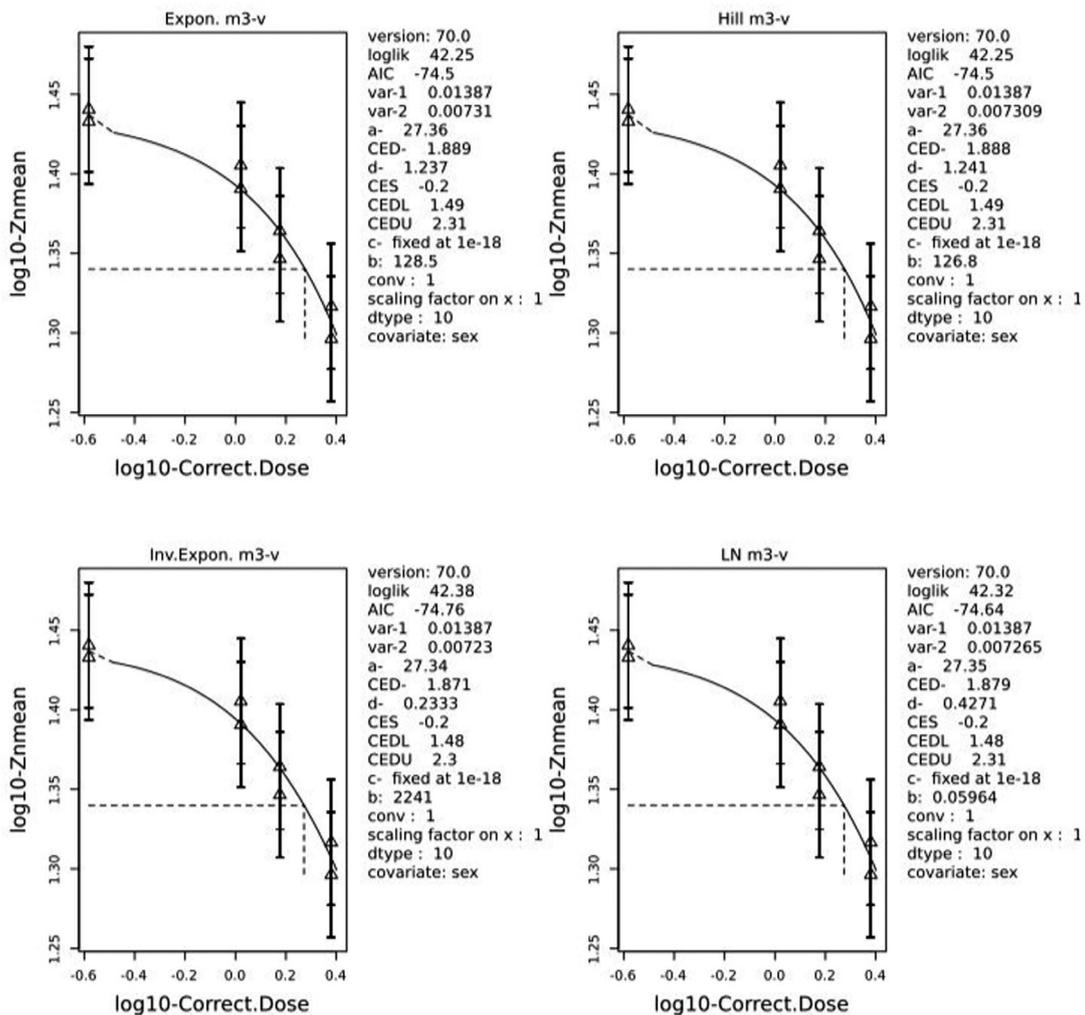
EXP	HILL	INVEXP	LOGN
0.24	0.24	0.27	0.25

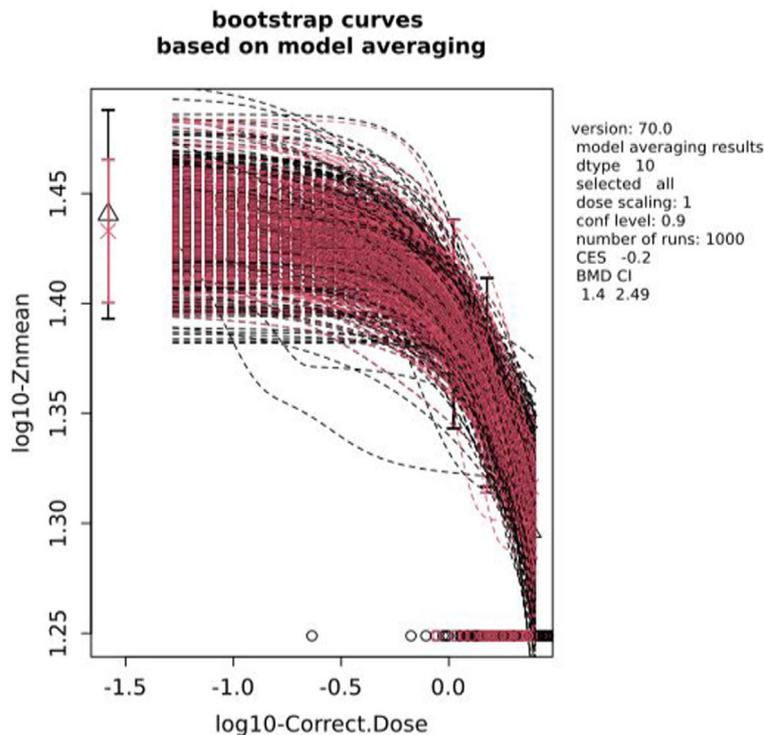
**Final BMD Values**

Endpoint (µmol/L)	subgroup	BMDL (g/kg bw per day)	BMDU (g/kg bw per day)
Zn level	M	1.36	2.49
Zn level	F	1.41	2.30

Confidence intervals for the BMD are based on 1,000 bootstrap data sets.

**Visualisation**





## Conclusions

The BMDL- and the BMDU values in males and females were close together and the confidence interval were both small. The BMDL was 1.36 g/kg bw per day (1,360 mg/kg bw per day) in males and 1.41 g/kg bw per day (1,410 mg/kg bw per day) in females and the BMDU 2.49 g/kg bw per day (2,490 mg/kg bw per day) in males and 2.30 g/kg bw per day (2,300 mg/kg bw per day) in females.

Hence, the Panel considered the lowest value of 1.36 g/kg bw per day (1,360 mg/kg bw per day), as the reference point derived from this study.

## Reference

Cole CR, Grant FK, Swaby-Ellis ED, Smith JL, Jacques A, Northrop-Clewes CA, Caldwell KL, Pfeiffer CM and Ziegler TR, 2010. Zinc and iron deficiency and their interrelations in low-income African American and Hispanic children in Atlanta. *The American Journal of Clinical Nutrition*, 91, 1027–1034. <https://doi.org/10.3945/ajcn.2009.28089>

## Appendix D – Benchmark Dose Modelling from the study in juvenile minipigs Glucose levels in blood

### Data Description

The endpoint to be analysed is: Glucose levels in blood (mean)

Data used for analysis are shown in Table D.1

The analysis considered the study 'Locust Bean Gum Piglet Adapted Formula Study in Juvenile Minipig' (Test Facility Study No. 78627, S. Ellemann-Laursen, 2020 and the final amended report No 1 of this study, 2022; documentation provided to EFSA n. 3,4,8).

**Table D.1:** Data used for the analysis

Correct.Dose (average g/kg bw per day)	Glucose.Mean (mmol/L)	Glu.SD	Glu.N	sex <sup>(a)</sup>
0.00	9.02	1.81	6	1
1.05	9.52	1.69	6	1
1.50	10.32	1.32	6	1
2.40	11.56	1.34	5	1
0.00	8.30	0.76	6	2
1.05	8.78	1.26	6	2
1.50	10.42	1.71	6	2
2.40	12.07	1.60	6	2

(a): 1 = males; 2 = females.

### Selection of the BMR

The BMR (benchmark response) used is a 30% change in mean response compared to the controls. The selection of the BMR of 30% for glucose is based on textbook reference values for postprandial glucose where an increase to 1.4 g/L is normal and an increase to 1.8 g/L indicates diabetes (corresponding to an increase of 28.6%).<sup>22</sup> As the piglets were non-fasting, the postprandial glucose levels are considered to provide the most relevant information on the changes in this parameter.

The BMD (benchmark dose) is the dose corresponding with the BMR of interest.

A 90% confidence interval around the BMD will be estimated, the lower bound is reported by BMDL and the upper bound by BMDU.

### Software Used

Results are obtained using the EFSA web-tool for BMD analysis, which uses the R-package PROAST, version 70.0, for the underlying calculations.

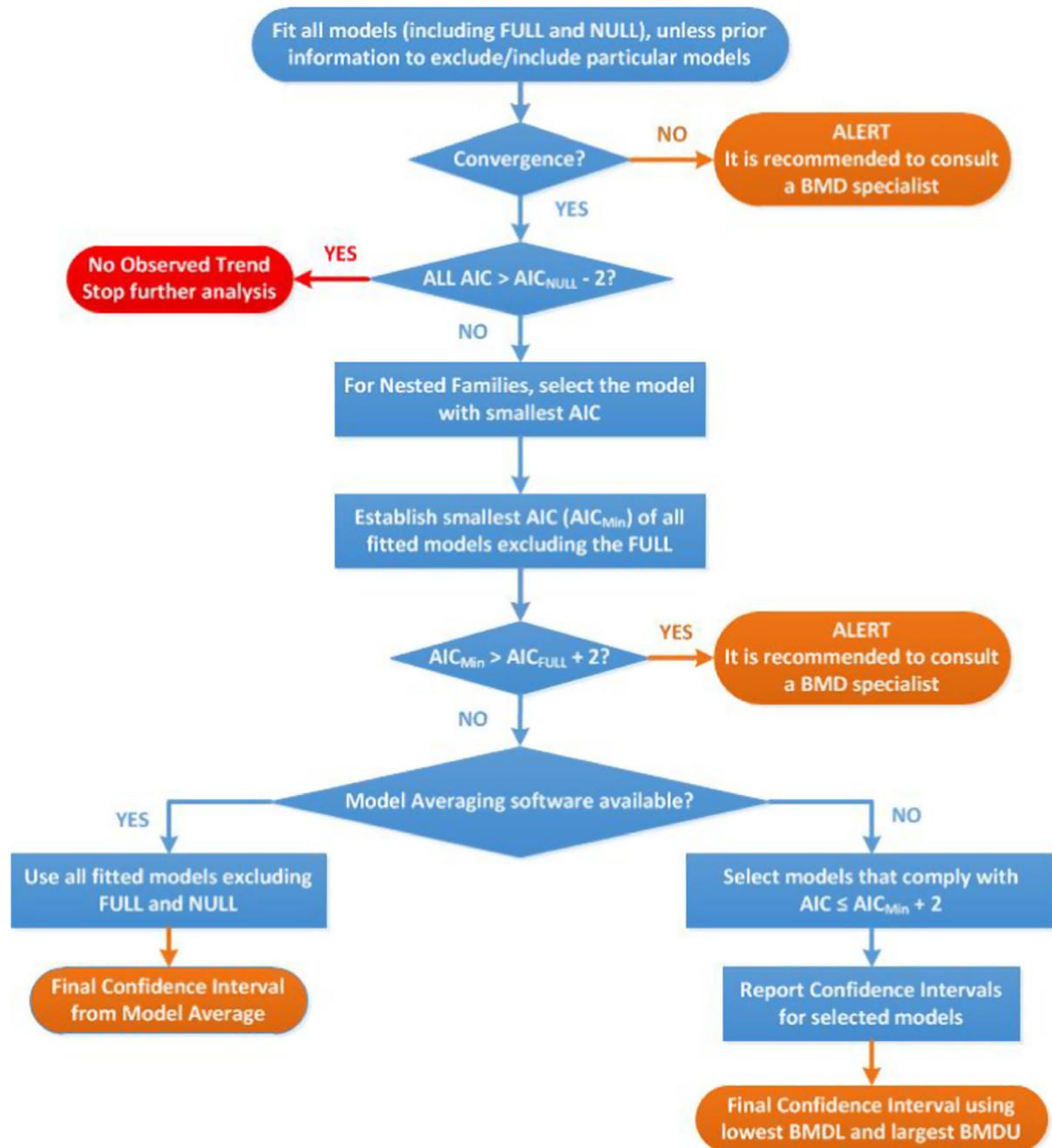
### Dose–response models

Default set of fitted models:

Model	Number of parameters	Formula
Null	1	$y = a$
Full	no. of groups	$y = \text{groupmean}$
Exp model 3	3	$y = a \cdot \exp(bx^d)$
Exp model 4	4	$y = a \cdot (c - (c-1)\exp(-bx^d))$
Hill model 3	3	$y = a \cdot \left(1 - \frac{x^d}{b^d + x^d}\right)$
Hill model 4	4	$y = a \cdot \left(1 - \frac{(c-1) \cdot x^d}{b^d + x^d}\right)$
Inverse Exponential	4	$y = a \cdot (1 + (c-1)\exp(-bx^{-d}))$
Log-Normal Family	4	$y = a \cdot (1 + (c-1)\Phi(\ln b + d \ln x))$

As a covariate is included in the analysis, these models will also be fitted assuming that some of the parameters [background response parameter (a), potency parameter (BMD) and/or variance (var)] depend on the subgroup defined by the covariate. Therefore, the number of parameters in each model might be larger than indicated in the table above.

**Procedure for selection of BMDL**



Flow chart for selection of BMDL

**Results**

Response variable: Glucose.Mean

**Fitted Models**

Model	converged	loglik	npar	AIC
full model	yes	27.59	9	-37.18
full-v	yes	27.92	10	-35.84
null model	yes	13.10	2	-22.20
null model-a	yes	13.15	3	-20.30
Expon. m3-	yes	26.03	4	-44.06

Model	converged	loglik	npar	AIC
Expon. m3-a	yes	26.24	5	-42.48
Expon. m3-b	yes	26.05	5	-42.10
Expon. m3-ab	yes	26.83	6	-41.66
Expon. m5-	yes	26.63	5	-43.26
Expon. m5-a	yes	26.84	6	-41.68
Expon. m5-b	yes	26.68	6	-41.36
Expon. m5-ab	yes	27.08	7	-40.16
Hill m3-	yes	26.03	4	-44.06
Hill m3-a	yes	26.24	5	-42.48
Hill m3-b	yes	26.05	5	-42.10
Hill m3-ab	yes	26.83	6	-41.66
Hill m5-	yes	26.49	5	-42.98
Hill m5-a	yes	26.70	6	-41.40
Hill m5-b	yes	26.50	6	-41.00
Hill m5-ab	yes	27.10	7	-40.20
Inv.Expon. m3-	yes	26.14	4	-44.28
Inv.Expon. m3-a	yes	26.35	5	-42.70
Inv.Expon. m3-b	yes	26.15	5	-42.30
Inv.Expon. m3-ab	yes	26.94	6	-41.88
Inv.Expon. m5-	yes	26.21	5	-42.42
Inv.Expon. m5-a	yes	26.43	6	-40.86
Inv.Expon. m5-b	yes	26.22	6	-40.44
Inv.Expon. m5-ab	yes	27.00	7	-40.00
LN m3-	yes	26.09	4	-44.18
LN m3-a	yes	26.30	5	-42.60
LN m3-b	yes	26.11	5	-42.22
LN m3-ab	yes	26.89	6	-41.78
LN m5-	yes	26.21	5	-42.42
LN m5-a	yes	26.42	6	-40.84
LN m5-b	yes	26.22	6	-40.44
LN m5-ab	yes	27.00	7	-40.00

### Estimated Model Parameters

#### EXP

estimate for var-: 0.01934  
 estimate for a-: 8.498  
 estimate for CED-: 2.072  
 estimate for d-: 1.572

#### HILL

estimate for var-: 0.01934  
 estimate for a-: 8.498  
 estimate for CED-: 2.071  
 estimate for d-: 1.576

#### INVEXP

estimate for var-: 0.01925  
 estimate for a-: 8.504  
 estimate for CED-: 2.053  
 estimate for d-: 0.2972

**LOGN**

estimate for var-: 0.01929  
 estimate for a-: 8.501  
 estimate for CED-: 2.061  
 estimate for d-: 0.5434

**Weights for Model Averaging**

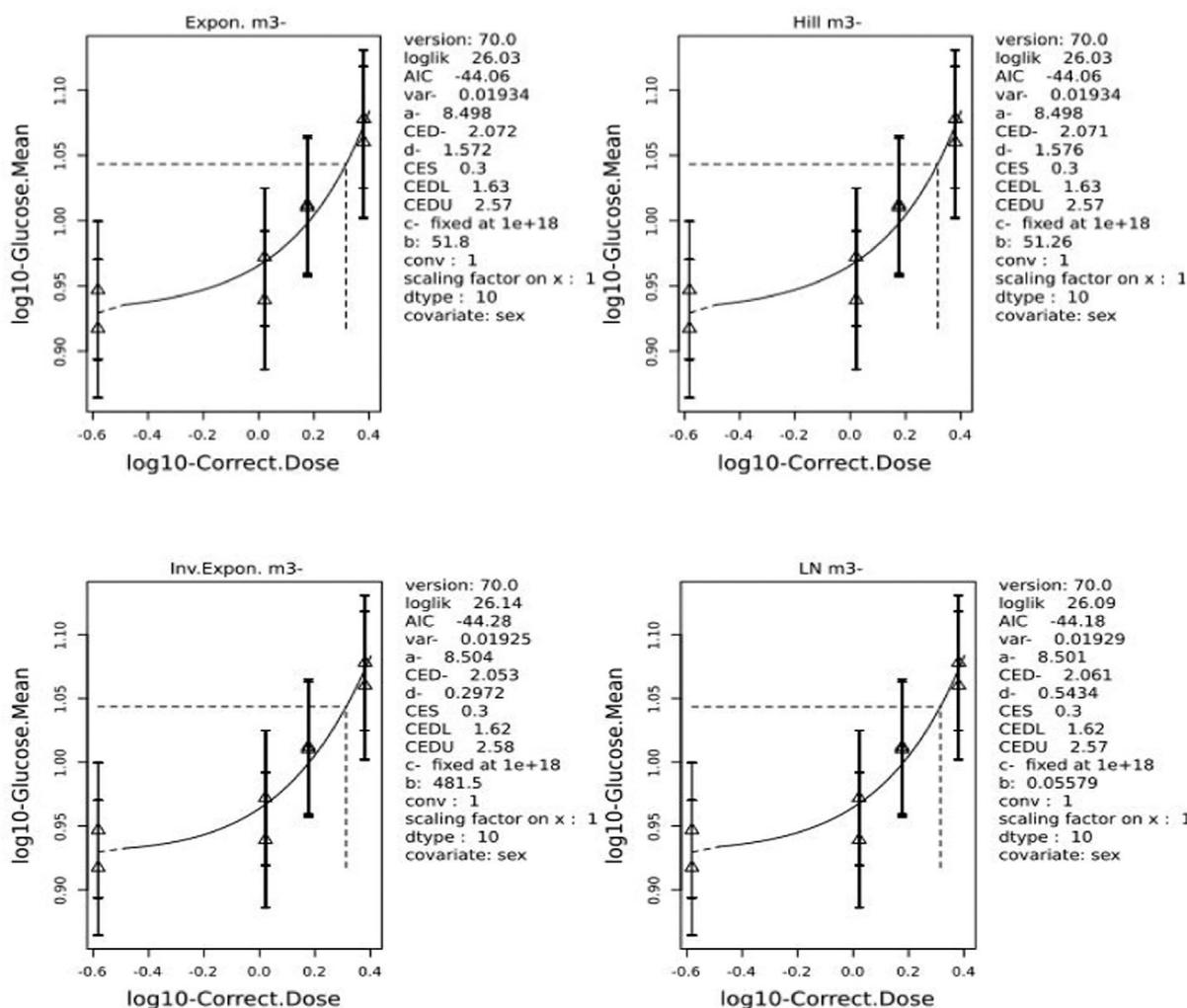
EXP	HILL	INVEXP	LOGN
0.24	0.24	0.27	0.25

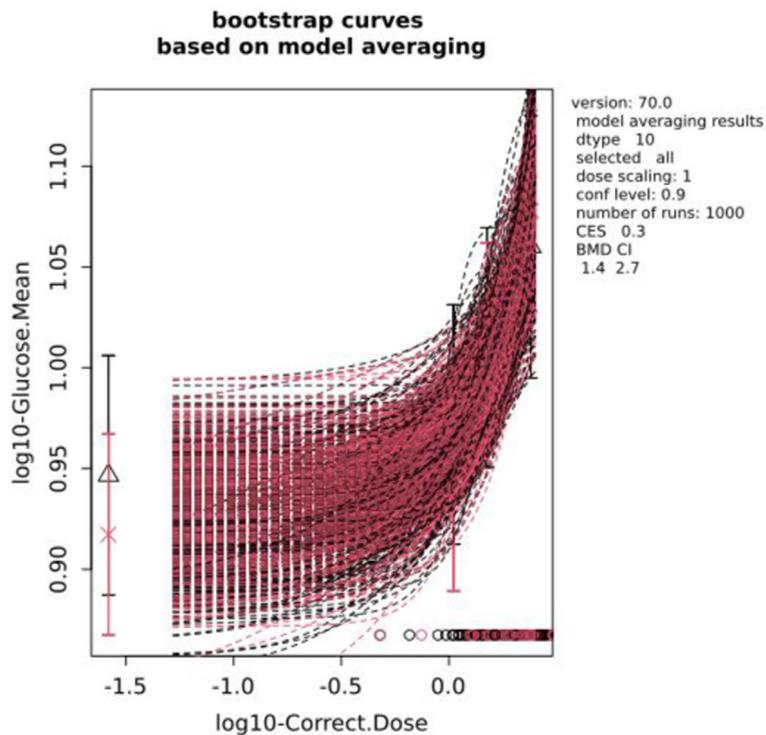
**Final BMD Values**

Endpoint (mmol/L)	Subgroup	BMDL (g/kg bw/day)	BMDU (g/kg bw/day)
Glucose level	M	1.45	2.70
Glucose level	F	1.53	2.48

Confidence intervals for the BMD are based on 1,000 bootstrap data sets.

**Visualisation**





## Conclusions

The BMDL- and the BMDU values in males and females were close together and the confidence interval were both small. The BMDL was 1.45 g/kg bw per day (1,450 mg/kg bw per day) in males and 1.53 g/kg bw per day (1,530 mg/kg bw per day) in females and the BMDU 2.70 g/kg bw per day (2,700 mg/kg bw per day) in males and 2.48 g/kg bw per day (2,480 mg/kg bw per day) in females.

Hence, the Panel considered the lowest value of 1.45 g/kg bw per day (1,450 mg/kg bw per day) as the reference point derived from this study.

## Appendix E – Estimation of the fraction of the concentration of toxic elements in locust bean gum (E 410) with respect to the regulatory maximum levels in the final food product for which the additive is used

The Panel estimated the fraction (%) of the concentration of the toxic elements lead (Pb) and cadmium (Cd) in locust bean gum (E 410) with respect to the regulatory maximum levels in the final product (formulae) as sold as laid down in Regulation (EC) No 1881/2006<sup>19</sup> considering:

- The current specification for Pb and Cd for locust bean gum (E 410) according to Regulation (EU) No 231/2012, 2 and 1 mg/kg, respectively.
- The lowest technically achievable levels of Pb and Cd in commercial unclarified locust bean gum, 0.5 and 0.25 mg/kg, respectively, as proposed by one IBO (Documentation provided to EFSA n. 1 and 3), see also Section 3.2.1
- The lowest technically achievable levels of Pb and Cd in commercial clarified locust bean gum, 1 and 0.1 mg/kg, respectively, as proposed by one IBO (Documentation provided to EFSA n. 5), see also Section 3.2.1
- The maximum permitted use level of locust bean gum E 410 in the final food of 10,000 mg/kg, the maximum use level reported by industry (10,000 mg/kg) and the mean use level reported by industry (4,347 mg/kg) for the uses in infant FSMP (FC 13.1.5.1) for infants below 16 weeks of age, see Section 3.3.2.1
- The range of maximum levels (ML) for Pb (0.01–0.02 mg/kg) and Cd (0.005–0.02 mg/kg) in formulae for infants as laid down in Regulation (EC) No 1881/2006<sup>19</sup>

The results of the calculations can be found in Tables E.1 and E.2 for lead and Tables E.3 and E.4 for cadmium.

**Table E.1:** Estimation of the fraction of the level of lead in locust bean gum (E 410) with respect to the regulatory maximum levels in the final product (liquid formulae for infants below 16 weeks of age)

Specification for toxic elements status	Lead (Pb) (mg/kg)	MPL or reported use level of E 410 in final product (mg/kg food) <sup>(a)</sup>	Resulting concentration of toxic element in final product (mg/kg food)	Maximum level in Reg. 1881/2006 (mg/kg)	Fraction of toxic element from FA on ML of final product ML (%)
Current EU specifications	2	10,000	0.02	0.010	200%
Current EU specifications	2	4,347	0.009	0.010	87%
Proposal IBO unclarified gum	0.5	10,000	0.005	0.010	50%
Proposal IBO unclarified gum	0.5	4,347	0.002	0.010	22%
Proposal IBO clarified gum	1	10,000	0.01	0.010	100%
Proposal IBO clarified gum	1	4,347	0.004	0.010	44%

(a): Maximum permitted Level (MPL) and maximum use level reported by the industry are both 10,000 mg/kg. The mean use level reported by the industry is 4,347 mg/kg.

**Table E.2:** Estimation of the fraction of the levels of lead in locust bean gum (E 410) with respect to the regulatory maximum levels in the final product (powder formulae for infants below 16 weeks of age)

Specification for toxic elements Status	Lead (Pb) (mg/kg)	MPL or maximum reported use level of food additive in final product (mg/kg food) (mg/kg) as reconstituted <sup>(a)</sup>	MPL or use level considering the dilution <sup>(b)</sup>	Concentration of toxic element in final product (mg/kg)	Maximum level in Reg. 1881/2006 (mg/kg) as sold	Fraction of toxic element from FA on ML of final product ML (%)
Current EU specifications	2	10,000	80,000	0.16	0.020	800%
Proposal IBO unclarified gum	0.5	10,000	80,000	0.04	0.020	200%
Proposal IBO clarified gum	1	10,000	80,000	0.08	0.020	400%

(a): The maximum levels of food additives set out in Annex II shall apply to the food as marketed, unless otherwise stated. By way of derogation from this principle, for dried and/or concentrated foods which need to be reconstituted the maximum levels shall apply to the food as reconstituted according to the instructions on the label taking into account the minimum dilution factor.

(b): Internal report on the harmonisation of dilution factors to be used in the assessment of dietary exposure, EFSA, 2018, available at <https://zenodo.org/record/1256085#.X89vU9hKiUk>.

**Table E.3:** Estimation of the fraction of the levels of cadmium in E 410 with respect to the regulatory maximum levels in the final product (liquid formulae for infants below 16 weeks of age, marketed as powder and manufactured from cow's milk proteins or from cow's milk protein hydrolysates)

Specification for toxic elements Status	Cadmium (mg/kg)	MPL or Use level of food additive in the final product (mg additive/kg food) <sup>(a)</sup>	Concentration of toxic element in final product (mg/kg)	Maximum level in Reg. 1881/2006 (mg/kg)	Fraction of toxic element from FA on ML of final product ML (%)
Current EU specifications	1	10,000	0.01	0.005	200%
Current EU specifications	1	4,347	0.004	0.005	87%
Proposal IBO unclarified gum	0.25	10,000	0.0025	0.005	50%
Proposal IBO unclarified gum	0.25	4,347	0.001	0.005	22%
Proposal IBO clarified gum	0.1	10,000	0.001	0.005	20%
Proposal IBO clarified gum	0.1	4,347	0.0004	0.005	9%

(a): Maximum permitted Level (MPL) and maximum use level reported by the industry are both 10,000 mg/kg. The mean use level reported by the industry is 4,347 mg/kg.

Considering the maximum level of 0.01 mg/kg for infant formulae 'marketed as liquid and manufactured from soya protein isolates, alone or in a mixture with cow's milk proteins protein', the fraction of toxic elements from the food additive on the ML of the final product would be half of the respective value in the last column.

**Table E.4:** Estimation of the fraction of the level of cadmium in locust bean gum (E 410) with respect to the regulatory maximum levels in the final product (powder formulae for infants below 16 weeks of age, marketed as powder and manufactured from cow's milk proteins or from cow's milk protein hydrolysates)

Specification for toxic elements Status	Cadmium (mg/kg)	MPL/Use level of food additive in final product (mg/kg) as reconstituted <sup>(a)</sup>	Use level considering the dilution <sup>(b)</sup>	Resulting concentration of toxic element in final product (mg/kg)	Maximum level in Reg. 1881/2006 (mg/kg)	Fraction of toxic element from FA as %age of final product ML (%)
current EU specifications	1	10,000	80,000	0.080	0.010	800%
Proposal IBO unclarified gum	0.25	10,000	80,000	0.020	0.010	200%
Proposal IBO clarified gum	0.1	10,000	80,000	0.008	0.010	80%

(a): The maximum levels of food additives set out in Annex II shall apply to the food as marketed, unless otherwise stated. By way of derogation from this principle, for dried and/or concentrated foods which need to be reconstituted the maximum levels shall apply to the food as reconstituted according to the instructions on the label taking into account the minimum dilution factor.

(b): Internal report on the harmonisation of dilution factors to be used in the assessment of dietary exposure, EFSA, 2018, available at <https://zenodo.org/record/1256085#.X89vU9hKiUk>.

Considering the maximum level of 0.02 mg/kg for 'infant formulae marketed as powder and manufactured from soya protein isolates, alone or in a mixture with cow's milk proteins protein', the fraction of toxic elements from the food additive on the ML of the final product would be half of the respective value in the last column.

Considering the results of the above estimations and the fact that the food additive is not the only potential source of toxic elements in the final food for infants, the Panel emphasises the need to reduce the specification limit values for lead and cadmium in Regulation (EU) no 231/2012.