Safety of mung bean protein as a novel food pursuant to Regulation (EU) 2015/2283

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Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on mung bean protein as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF, which is the subject of the application, is mung bean protein extracted from seeds of the plant *Vigna radiata*. The NF is proposed to be used as a food ingredient added to ‘protein products, excluding products covered in category 1.8’. The target population is the general population. The maximum estimated intake of the NF is 758 and 260 mg/kg body weight (bw) per day in children and adults, respectively. The major constituents of this NF are protein (~85%), fat (3–4%) and moisture (3–5.5%). The NF is rich in protein which is well digestible and provides sufficient amounts of most essential amino acids but only limited amounts of sulfur-containing amino acids. The Panel notes that the cumulative exposure to the minerals analysed does not raise concern. The reported values for the levels of antinutritional factors in the NF are comparable to those in other foodstuffs. The Panel considers that taking into account the composition of the NF and the proposed conditions of use, consumption of the NF is not nutritionally disadvantageous. No toxicological studies with the NFs were provided by the applicant; however, the Panel considers that no toxicological studies are required on this NF. This NF has the potential capacity to sensitise individuals and to induce allergic reactions in individuals allergic to soybean, peanuts, lupin and to birch pollen. The Panel considers that the NF, i.e. mung bean protein, is safe at the proposed conditions of use.

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1. Introduction

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

On 10 March 2020, the company Eat Just, Inc submitted a request to the Commission in accordance with Article 10 of Regulation (EU) No 2015/2283 to place on the EU market mung bean protein. Mung bean protein is isolated from dry mung bean seeds by a sequence of processing steps followed by drying. Mung bean protein is intended to be used as an ingredient in a variety of foods.

In accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission asks the European Food Safety Authority to provide a scientific opinion on mung bean protein.

1.2. Additional information

It is noted that the applicant refers to a Generally Recognized As Safe (GRAS) affirmation on mung bean protein and a notification to the U.S. FDA on the conclusions. The FDA GRAS on this product was published in 2017.1

2. Data and methodologies

2.1. Data

The safety assessment of this NF is based on data supplied in the application and information submitted by the applicant following an EFSA request for supplementary information.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469.2

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of a NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data, (including both data in favour and not in favour) that are pertinent to the safety of the NF.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data requested by the applicant to be protected comprise analytical data on phytic acid, lectins, trypsin inhibitors, cyanogenic glycosides and tannins.

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of the Commission Implementing Regulation (EU) 2017/2469.

Additional information which was not included in the application was retrieved by literature search following a search strategy and standard operating procedure as described by UCT Prague (2020).

This assessment concerns only the risks that might be associated with consumption of the NF under the proposed conditions of use and is not an assessment of the efficacy of the NF with regard to any claimed benefit.

3. Assessment

3.1. Introduction

The novel food (NF), which is the subject of the application, is mung bean protein extracted from seeds of the plant *Vigna radiata* by several processing steps followed by pasteurisation and spray drying. The NF is proposed to be used as a food ingredient added to 'protein products, excluding

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1 https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=684
2 Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 64–71.
products covered in category 1.8\(^3\). The target population is the general population, and it is not intended to be used in infant formulae and follow-on formulae.

The applicant indicates that, as defined by Regulation (EU) 2015/2283, Article 3 (iv), the NF falls under the category ‘food consisting of, isolated from or produced from plants or their parts, except when the food has a history of safe food use within the Union and is consisting of, isolated from or produced from a plant or a variety of the same species obtained by:

- traditional propagating practices which have been used for food production within the Union before 15 May 1997; or
- non-traditional propagating practices which have not been used for food production within the Union before 15 May 1997, where those practices do not give rise to significant changes in the composition or structure of the food affecting its nutritional value, metabolism or level of undesirable substances’.

The assessment of the dossier is based on the data presented by the applicant in the dossier for authorisation of the NF in the context of Regulation (EU) 2015/2283. It is also noted that the applicant refers to GRAS notice on this NF from FDA\(^4\). Furthermore, the Food Standards Agency\(^5\) has previously published a report on the status of this NF pursuant to Regulation (EC) No 258/97.

### 3.2. Identity of the NF

The NF is mung bean protein powder extracted from the seeds of the plant *Vigna radiata*. The plant belongs to the plant family Fabaceae. The most relevant seed storage proteins in mung bean are globulins, mainly vicilin type (8S, about 90%), legumin type (11S, about 8%) and basic 7S type (~ 3%) proteins (Mendoza et al., 2001). Proteins were characterised by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) under reducing and non-reducing conditions and the pattern is comparable with that reported by Rahma et al. (2000).

The applicant states that the raw materials used for the extraction of the protein are typically cultivated in China, India and Tanzania.

### 3.3. Production process

According to the information provided, the NF is produced in line to Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Points (HACCP) principles.

Mung bean protein is extracted from seeds using the same mechanical steps employed for protein extraction of other seeds such as soybean, pea, rapeseed or lupin. Prior to milling, the beans are heated at high temperatures for few minutes to assure consistent moisture and to reduce undesirable volatile flavours. After milling, mung bean protein is extracted in aqueous solution at a slightly alkaline pH and low concentration of NaCl. Fibre and starch are separated by decantation. Afterwards, the protein is precipitated from the extract by lowering the pH by means of citric acid. The precipitate is then re-dispersed in water and neutralised, followed by pasteurisation and spray drying. The mung bean protein, which is the NF, is a white and dry powder.

The Panel considers that the production process is sufficiently described and does not raise safety concerns.

### 3.4. Compositional data

The NF is a dry, white powder and the major constituents are protein (88–91%), fat (3.4%) and moisture (3–5.5%).

In order to confirm that the manufacturing process is reproducible and adequate to produce on a commercial scale a product with certain required characteristics, the applicant provided analytical information for six independent batches of the NF (Table 1).

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\(^3\) This category includes protein analogues or substitutes for standard products, such as meat, fish or milk; including gelatine and unflavoured soy drinks.

\(^4\) https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=684

\(^5\) https://ec.europa.eu/food/system/files/2019-02/novel-food_consult-status_mung-bean-protein.pdf
The applicant also provided detailed analyses for minerals (Table 2), heavy metals (Table 3), microbial quality (Table 4), and antinutritional factors, phenolics and cyanogenic glycosides (Table 5). The applicant also analysed the amino acid composition of the NF (Appendix A).

The applicant compared the concentrations of antinutritional factors, phenolics and cyanogenic glycosides in whole mung bean flour (Appendix B) with those in the NF (Table 5). The Panel notes that lectins, phytic acid and tannins are higher in the NF than in the mung bean flour which is expected owing to their association with the protein fraction.
The applicant performed a multiresidue pesticide screen (method EN15662/CFIA PMR-006). All compounds were below the limit of detection of 0.005 ppm.

Information was provided on the accreditation of the laboratories that conducted the analyses presented in the application, with the exception of the measurements of antinutritional factors and cyanogenic glycosides which were performed in-house.

The Panel considers that the information provided on the composition is sufficient for characterising the NF.

### 3.4.1. Stability

The applicant performed stability tests with five independently produced batches of the NF. The tests were carried out at 20°C in a dry environment. The batches were analysed for microbial and amino acid composition. The outcome of the study revealed no microbial growth and no relevant changes in amino acid composition of the protein powders after the storage period of 11–13 months.

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**Table 4:** Batch-to-batch analysis of microbial data in the NF

| Parameter (Unit) | Batches | Method of analysis |
|------------------|---------|-------------------|
| Aerobic plate count (CFU/g) | #1 50 | AOAC 966.23 |
| | #2 < 10 | |
| | #3 90 | |
| | #4 20 | |
| | #5 2,800 | |
| | #6 < 10 | |
| Coliforms (Petrifilm) (CFU/g) | < 10 | AOAC 991.14 |
| Escherichia coli (Petrifilm) (CFU/g) | < 10 | AOAC 991.14 |
| Mesophilic aerobic spores (CFU/g) | 8 | CMMEF, 5th ed. |
| Yeasts (CFU/g) | < 10 | FDA-BAM, 7th ed. |
| Moulds (CFU/g) | 50 | FDA-BAM, 7th ed. |
| Genus Listeria (not detected in 25 g) | Not detected | AOAC 2004.06 |
| Salmonella (not detected in 375 g) | Not detected | AOAC 2004.03 |

**Table 5:** Batch-to-batch analysis of antinutritional factors, phenolics and cyanogenic glycosides in the NF

| Parameter (Unit) | Batches | Method of analysis |
|------------------|---------|-------------------|
| Lectin (HAU/g) | #1 120 | Lectin testing by haemagglutination (TES-AC358) |
| | #2 120 | |
| | #3 120 | |
| | #4 120 | |
| Phytic acid (mg/g) | #1 13.3 | Phytic acid (PHYT_S) |
| | #2 12.6 | |
| | #3 12.8 | |
| | #4 11.1 | |
| | #5 13.4 | |
| Trypsin inhibitors (TIU/mg) | #1 5.88 | Trypsin inhibitor (TRYP_IN_S) |
| | #2 4.35 | |
| | #3 5.41 | |
| | #4 2.91 | |
| | #5 3.55 | |
| Tannins | 1.52 | 
| Total phenolic content (mg/g dw) | 5.85 | 
| | 1.02 | 
| | 8.59 | 
| | 1.01 | 
| | 6.81 | 
| | 1.08 | 
| | 6.46 | 
| | 0.97 | 
| | 6.84 | 
| Cyanogenic glycosides (μg/g) | < LOD | LC-MS/MS |
| | < LOD | |
| | < LOD | |
| | < LOD | |
| | < LOD | |
| | < LOD | |

CFU: colony forming units; AOAC: Association of Official Agricultural Chemists; CMMEF: compendium of methods for the microbiological examination of foods; FDA-BAM: U.S. Food and drug administration - Bacteriological analytical manual.

dw: dry weight; HAU: Haemagglutination Units; TIU: Trypsin Inhibitor Units; DMAC: dimethylaminocinnamaldehyde; LOD: limit of detection of 5 ppb; LC–MS/MS: liquid chromatography with tandem mass spectrometry.

The applicant performed a multiresidue pesticide screen (method EN15662/CFIA PMR-006). All compounds were below the limit of detection of 0.005 ppm.

Information was provided on the accreditation of the laboratories that conducted the analyses presented in the application, with the exception of the measurements of antinutritional factors and cyanogenic glycosides which were performed in-house.

The Panel considers that the information provided on the composition is sufficient for characterising the NF.
Therefore, the applicant proposes a shelf life of the NF of 12 months. The applicant did not provide stability data for representative processed foods.

The Panel considers that the data provided sufficient information with respect to the stability of the NF.

3.5. Specifications

The specifications of the NF are indicated in Table 6.

| Table 6: Specifications of the NF |
|-----------------------------------|
| **Description:** Protein isolate extracted from mung bean flour |
| **Source:** Mung bean, *Vigna radiata* |
| **Parameter** | **Specification** |
| Moisture | Max 6% |
| Protein (w/w) | Min 84% |
| Ash (w/w) | Max 6.0% |
| Fat (w/w) | Max. 5.5% |
| Carbohydrate (w/w) | Max 5.0 by calculation |
| **Microbiological** | |
| Aerobic plate count | < 5,000 CFU/g |
| *Escherichia coli* | < 10 CFU/g |
| Coliforms | < 100 CFU/g |
| Yeasts (CFU/g) | < 100 CFU/g |
| Moulds (CFU/g) | < 100 CFU/g |
| *Listeria monocytogenes* | Not detected in 25 g |
| *Salmonella* spp. | Not detected in 25 g |

CFU: colony forming units; w/w: weight per weight.

The applicant proposed a specification limit for aerobic plate count as < 10,000 CFU/g. The Panel notes that considering the NF production process and compositional analyses of five batches, a lower specification limit could be met. A similar NF is authorised by Commission Implementing Regulation (EU) 2021/1206, where the microbiological criteria for aerobic plate count are < 5,000 CFU/g.

The Panel notes that aerobic plate count is an indicator of hygiene and considers that this quality parameter ultimately also contributes to the safety of a NF.

The Panel considers that the information provided on the specifications of the NF is sufficient and does not raise safety concerns.

3.6. History of use of the NF and/or of its source

3.6.1. History of use of the source

Mung bean plants have been consumed by humans since long ago (Fuller and Harvey, 2006). The main parts consumed are seeds and sprouts of mung bean, the safety of which has been previously discussed (Shanmugasundaram et al., 2010; Tang et al., 2014). The consumption of mung bean varies depending on the geographic region. For example, in India, mung bean is used in sweets, snacks and savoury items (Adsule et al., 1986). In other parts of Asia, it is used in cakes, sprouts, noodles and soups (Tang et al., 2014). In America and Europe, it is mainly used as fresh bean sprouts.

The consumption of mung beans as such in the US is in the order of 22–29 g/capita per year (USDA, 2015), while the consumption in some areas of Asia can be as high as 2 kg/capita per year (Vijayalakshmi et al., 2003).

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6 Commission Implementing Regulation (EU) 2021/120 of 2 February 2021, authorising the placing on the market of partially defatted rapeseed powder from *Brassica rapa* L. and *Brassica napus* L. as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council and amending Commission Implementing Regulation (EU) 2017/2470.
3.6.2. History of use of the NF

There is no history of safe use of mung bean protein in the EU prior to 15 May 1997. According to the applicant, mung bean protein is authorised as a novel food ingredient in Asia and US since 2017.

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

The target population proposed by the applicant is the general population. As the NF is intended to be used as an ingredient in standard food categories, the NF can be consumed by any population group. Therefore, the safety data and the exposure assessment shall cover all population groups, according to Commission Implementing Regulation (EU) 2017/2469, article 5(6).

3.7.2. Proposed uses and use levels

The NF is proposed to be used as an ingredient in food products, at the maximum use level as indicated in Table 7. These food products are reported in Table 7.

Table 7: Food categories in FAIM Food categories and maximum use levels intended by the applicant

| Food category | Proposed maximum use level (g NF/kg food) |
|---------------|------------------------------------------|
| 12.9          | Protein products, excluding products covered in category 1.8* |
|               | 200                                      |

*: Category 12.9 includes protein analogues or substitutes for standard products, such as meat, fish or milk; including gelatine and unflavoured soy drinks. Category 1.8 includes dairy analogues, including beverage whiteners.

(a): Food categories according to Annex II to Regulation (EC) No 1333/2008, and used in the FAIM tool.

3.7.3. Anticipated intake of the NF

The estimated daily intake of the NF for each population group can be found in Table 8, as derived from the FAIM tool, which is a tool for estimating chronic dietary exposure to food additives. The FAIM tool is based on individual data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011).

The estimated daily intake of the NF for each population group from each EU dietary survey is available in the excel file annexed to this scientific opinion (under supporting information).

Table 8: Intake estimate resulting from the use of the NF as an ingredient in the intended food categories at the maximum proposed use levels per age class (mg/kg bw per day)

| Population group          | Age (years) | Min average | Max average | Min 95th | Max 95th |
|---------------------------|-------------|-------------|-------------|----------|----------|
| Infants                   | < 1         | 0           | 31.9        | 0        | 0        |
| Young children(3)         | 1–< 3       | 0           | 581.1       | 0        | 64.3     |
| Other children            | 3–< 10      | 0           | 176.9       | 0        | 757.6    |
| Adolescents               | 10–< 18     | 0           | 16.8        | 0        | 71.3     |
| Adults                    | 18–< 65     | 0.4         | 31.7        | 0        | 259.7    |
| Elderly and very elderly  | > 65        | 0.1         | 19.7        | 0        | 66.7     |

bw: body weight.

(a): Referred as ‘toddlers’ in the EFSA Food Additives Intake Model 2.0 (FAIM) tool (https://www.efsa.europa.eu/en/applications/food-improvement-agents/tools).

3.7.4. Combined intake from the NF and other sources

Mung beans are mainly consumed in Asia. In the EU, they are used at a lesser extent and mainly as mung bean sprouts (see Section 3.6.1).
3.7.5. Precautions and restrictions of use

The applicant indicated that the NF is not suitable as the sole source of dietary protein.

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

No ADME data have been provided for the NF. This NF is mainly composed by protein (min. 84%). Mung bean protein is a globular protein similar to soy, pea, bean, lupin, peanut or other legume proteins. The applicant performed a literature search concerning ADME of mung bean protein. The references identified mainly addressed nutritional aspects of mung bean protein which are discussed in Section 3.9.

3.9. Nutritional information

The applicant provided a nutritional analysis of the NF. The major components of the NF are protein, fat, carbohydrates and salt. The nutritional profile of the NF can be found in Table 9.

Table 9: Nutritional profile of the NF

| Description | Protein isolate extracted from mung bean flour |
| Source | Mung bean, Vigna radiata |
| **Nutritional information** | Per 100 g of NF |
| Energy* | 1,690 kJ |
| | 406 kcal |
| Fat | 5.5 g |
| Carbohydrates | 5 g |
| Dietary fibre | 0 g |
| Protein | 84.0 g |
| Salt** | 2.2 g |

*: Calculated according to Regulation (EU) No 1169/2011: fat 9 kcal/g; carbohydrates 4 kcal/g, dietary fibre 2 kcal/g, protein 4 kcal/g. **: Salt content calculated as NaCl according to Regulation (EU) No 1169/2011: 2.5 × sodium content.

To address the nutritional quality of the NF, the applicant studied the capacity of the protein in the NF to satisfy the requirements for essential amino acid and the metabolic needs for amino acids and nitrogen. Furthermore, antinutrients and minerals in the NF were also assessed.

In relation to the protein quality of the NF, the applicant provided data on the amino acid composition of the protein of the NF, the amino acid score (Table 10) and digestibility of the protein.

Table 10: Calculated amino acid scores for the NF

| Amino acid | Indispensable amino acids in the NF (mg/g protein)¹ | Scoring pattern (indispensable amino acid reference profiles) for children aged 3–10 years² (mg/g protein) | Calculated AAS (%) of the NF using amino acid reference profile for children aged 3–10 years² |
|------------|-----------------------------------------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| Histidine  | 28.7                                          | 16                                                                                              | 1.79                                                                                             |
| Isoleucine | 48.6                                          | 31                                                                                              | 1.57                                                                                             |
| Leucine    | 85.8                                          | 61                                                                                              | 1.41                                                                                             |
| Lysine     | 70.7                                          | 48                                                                                              | 1.47                                                                                             |
| Methionine + cysteine | 16.7 | 24                                                                 | 0.68                                                                                             |
| Tyrosine + phenylalanine | 101.5 | 41                                                                 | 2.48                                                                                             |
| Threonine  | 28.1                                          | 25                                                                                              | 1.12                                                                                             |
| Tryptophan | 9.5                                           | 6.6                                                                                             | 1.44                                                                                             |
| Valine     | 54.7                                          | 40                                                                                              | 1.37                                                                                             |

AAS: amino acid score.
1: Amino acid content in mg/g protein based on the analytical results from the NF (Appendix A).
2: As described by EFSA and WHO (WHO, 2007; EFSA NDA Panel, 2012).
As presented in Table 10, the NF provides reasonable amounts of the indispensable (essential) amino acids with the exception of the sulfur-containing amino acids methionine and cysteine. This is in line with the fact that the 8S vicilin-like globulin, which does not contain any cysteine, is the main protein fraction in the mung bean protein.

The applicant assessed protein digestibility and quality by Protein Digestibility Corrected Amino Acid Score (PDCAAS) and conducted an in vivo faecal digestibility study in rats. To assess whether processing affects the NF digestibility, the protein isolate was used as such and in prepared (cooked) form. Male Sprague-Dawley rats (4/group) were fed 15 g/day test diets containing 10% protein (casein as positive control, uncooked or cooked NF), and other nutrients including vitamins and minerals fulfilling the animals’ requirements for energy and other nutrient intakes, for nine consecutive days. The control group received a protein-free diet formulated to match the nutrient and energy content of the test diets except for protein which was replaced with corn starch.

The true faecal digestibility of the uncooked NF was found to be 95.7% and 97.0% and 94.8% and 97.3% for the cooked protein.

The resulting PDCAAS values were calculated to be 0.638 and 0.635 for uncooked NF and 0.580 and 0.598 for the cooked NF, when using the reference scoring pattern from WHO/FAO/UNU, 2007 (EFSA, NDA Panel, 2012). The Panel notes that mung bean protein is well digestible (Moughan et al., 2012; Rutherfurd et al., 2012, 2015; Devi et al., 2018; Kashyap et al., 2019; Shivakumar et al., 2019). It provides sufficient amounts of most essential amino acids but only limited amounts of sulfur-containing amino acids, and for this reason, the PDCAAS of the NF is lower than that for other legume proteins (Hughes et al., 2011; Guillin et al., 2021; Rutherfurd et al., 2015). Based on the highest (max. average for infants and young children, 95th percentile for other children, adolescents and adults) intake levels of the NF (Section 3.7.3) with a protein content of up to 90.6% (according to results of batch analyses in Table 1), corresponding protein intake from mung bean protein per kg body weight and day could amount to 0.029 g for infants, 0.53 g for young children, 0.69 g for other children, 0.06 g for adolescents and 0.24 g for adults. These intakes would correspond to about 2.2%, 46–59%, 75–80%, 7.2–7.8%, and 28% of the dietary reference values (DRVs) for protein for infants, young children, other children, adolescents and adults, respectively. Considering that the NF is not intended to be the sole source of dietary protein, as it is intended to be integrated into a varied and mixed diet, and that the average protein intake in EU population is high and frequently above DRVs (EFSA NDA Panel, 2012), the risk for this situation to occur is deemed low.

The applicant analysed the mineral content of the NF (see Section 3.5) and compared the 95th percentile of NF intake in different population groups with available upper levels, also considering the background intake from the diet. The Panel notes that the cumulative exposure to the minerals analysed does not exceed the upper levels (UL) for any of the population groups except for magnesium (EFSA NDA Panel, 2015; Appendix C.1). However, the UL for magnesium only applies to readily dissociable magnesium salts and compounds like MgO in food supplements, water or added to foods, and does not include magnesium naturally present in foods and beverages. For sodium (for which there is no UL), the cumulative intake is above the safe level of intake (EFSA NDA Panel, 2019; Appendix C.2). Furthermore, the Panel notes that the UL for magnesium and the safe level of intake for sodium may be already exceeded by the intake from the background diet, and therefore that the contribution from the NF is small and does not raise concern (EFSA NDA Panel, 2015; EFSA NDA Panel, 2019).

Antinutritional factors (phytic acid, tannins, cyanogenic glycosides, trypsin inhibitors and lectin) in mung bean were also analysed by the applicant (see Section 3.4).

For phytic acid, the levels determined in the NF (Table 5) are comparable with the contents in other foods, plant seed protein isolates or plant-derived foodstuffs as previously described (EFSA NDA Panel, 2013). The applicant also provided analytical data of the NF content of tannins (see Section 3.4). The total daily intake of polyphenols for subjects consuming a Mediterranean diet was reported to be as high as 2.5–3 g/day (EFSA NDA Panel, 2013). Therefore, a high intake of NF could result in daily intakes of phenolic compounds (including tanning) below 50 mg for adults, which would not substantially increase the total intake of polyphenols from the diet.

For cyanogenic glycosides, values were below limit of detection in the NF (Table 5).
In the case of trypsin inhibitors, Avilés-Gaxiola et al. (2018) reported trypsin inhibitor activity (U/mg) of selected legumes seeds which ranged from 1 to 5 U/mg in, e.g. *Vigna radiata*, *Vigna mungo*, *Vicia faba*, *Pisum sativum*, to ~ 90 U/mg in soybean. Finally, for lectins, the analytical value obtained for haemagglutination by lectin was 120 HAU/g which is more than 10 times lower compared to the mean lectin content reported for soybean seed. The Panel notes that the NF is produced from mung bean after heat treatment at high temperatures for a few minutes which is expected to substantially inactivate trypsin inhibitors and lectins.

The Panel considers that taking into account the composition of the NF and the proposed conditions of use, consumption of the NF is not nutritionally disadvantageous.

### 3.10. Toxicological information

The Panel notes that no toxicological studies with the NFs were provided. Instead, the applicant referred to the fact that:

- mung beans are widely consumed in Asia and they are also consumed in the US and the EU;
- mung bean protein in the NF is not chemically modified as it is extracted by mechanical means; and
- mung bean protein is structurally related to seed storage proteins in other legumes such as soy, lupin, and pea.

The applicant performed a literature search with respect to the toxicity of mung bean protein. Yao et al. (2015) carried out a 90-day subchronic oral toxicity study in rats of bruchid beetle-resistant mung bean (650 g/kg diet). No adverse effects were observed in rats consuming bruchid-resistant mung bean when compared to rats consuming conventional cultivars and the control diet. However, given that it was mung bean flour the conclusions of this study might not be directly extrapolated to the toxicity of the NF.

Taking into account the nature of the NF and the elements described above, the Panel considers that no toxicological studies are required on the NF.

#### 3.10.1. Human data

The Panel notes that there are no human studies conducted with the NF. Following a literature search performed by the applicant, no human studies were identified on the safety of mung bean protein. A recent clinical study was retrieved from the literature (Bartholomae et al., 2019), but it focused on investigating potential beneficial effects of mung bean protein which are outside the scope of this scientific opinion.

### 3.11. Allergenicity

Mung bean is a legume seed and allergenicity of legume seeds is well known, ranging from local skin reactions to anaphylaxis (reviewed by Nwaru et al., 2014). Legume seeds such as peanut, soybean and lupin are considered common allergenic foods by European regulation. The major seed storage proteins of mung beans are globulins, mainly vicilin-type, legumin-type and basic-type (Mendoza et al., 2001). Mittag et al. (2005) studied food allergy to mung bean and identified the Vg r1 allergen, a pathogenesis-related protein, synthesised in the seedlings. Subsequently, Misra et al. (2011) identified four clinically relevant allergens (Vg r2, Vg r3, Vg r4 and Vg r5) in mung bean seeds showing pepsin resistance and IgE-binding capacity against sensitised human and mice sera. Finally, Guhl et al. (2014) described the Vg r 6 allergen, the cytokinin-specific binding protein which cross-reacts with Bet v 1-related allergens and binds IgE from birch pollen allergic patients’ sera. Most of the identified allergens originate from globular storage proteins, but profilins and pathogenesis-related proteins in seedlings were also shown to have an IgE-binding capacity (Sanchez-Monge et al., 2004; Verma et al., 2013).

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9 Regulation (EU) No 1169/2011 of the European parliament and of the council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.
Data on clinical relevance and incidence of allergy to mung bean are scarce and mung-bean allergic individuals are mainly identified in India, where the beans are commonly consumed (Misra et al., 2011). Furthermore, cross-reactivity among legume proteins have been reported (Jensen et al., 2008; Szymkiewicz and Chudzik-Kozłowska, 2013; Verma et al., 2013). Homologies between mung bean proteins and those of soybean, peanut and lupin, calculated by the applicant using the BLAST program in the database UniProtKB reference proteomes plus SwissProt were shown to be higher than 50%.

The applicant did not perform any test to assess the allergenicity of the NF. However, considering the information above, this NF has the potential capacity to sensitise individuals and to induce allergic reactions (co-sensitisation or cross-reactivity) in individuals allergic to soybean, peanut, lupin as well as to birch pollen.

4. Discussion

The NF, which is the subject of the application, is mung bean protein extracted from seeds of the plant *Vigna radiata* by several processing steps followed by pasteurisation and spray drying. Mung beans have been consumed by humans since long ago and the main materials consumed are seeds as well as sprouts of mung bean. The safety of mung bean protein for human consumption has previously been assessed by the U.S. FDA and no safety concerns were identified under the conditions of use.

The NF is proposed to be used as a food ingredient added to ‘protein products, excluding products covered in category 1.8’. The target population is the general population, and it is not intended to be used in infant formulae and follow-on formulae.

The maximum estimated intake of the NF is 758 and 260 mg/kg bw per day in other children and adults, respectively. The major constituents of this NF are protein (~85%), fat (3–4%) and moisture (3–5.5%). The protein in the NF is well digestible and provides sufficient amounts of most essential amino acids but only limited amounts of sulfur-containing amino acids. The Panel notes that the cumulative exposure to the minerals analysed does not raise concern. The reported values for the levels of antinutritional factors in the NF are comparable to those in other foodstuffs.

No toxicological studies with the NFs were provided by the applicant. The Panel considers that no toxicological studies are required on the NF because (i) mung beans are widely consumed in Asia and they are also consumed in the US and the EU; (ii) mung bean protein in the NF is not chemically modified as it is extracted by mechanical means; and (iii) mung bean protein is structurally related to seed storage proteins in other legumes such as soy, lupin, and peanut.

Considering the information provided, this NF has the potential capacity to sensitise individuals and to induce allergic reactions (co-sensitisation or cross-reactivity) in individuals allergic to soybean, peanut, lupin as well as to birch pollen.

5. Conclusions

The Panel concludes that the NF, mung bean protein, is safe under the proposed conditions of use.

5.1. Protection of Proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the data claimed as proprietary by the applicant (analytical data on phytic acid, lectins, trypsin inhibitors, cyanogenic glycosides and tannins).

**Steps taken by EFSA**

1) On 6/8/2020 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of mung bean protein as a novel food. Ref. Ares (2020) 4112438.

2) On 6/8/2020, a valid application on mung bean protein, which was submitted by Eat Just, Inc. (JUST), was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2020/1651) and the scientific evaluation procedure was initiated.

3) On 20/11/2020 and on 25/6/2021, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 26/3/2021 and 23/8/2021, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.

5) During its meeting on 14/9/2021, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of mung bean protein as a NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| AAS          | Amino acid score |
| ADME         | Absorption, distribution, metabolism, excretion |
| AQAC         | Association of Official Agricultural Chemists |
| Aw           | Water activity |
| BAM          | Bacteriological analytical manual |
bw | body weight  
---|-------------
CFU | colony forming unit  
CMMEF | compendium of methods for the microbiological examination of foods  
DMAC | dimethylaminocinnamaldehyde  
DRVs | dietary reference values  
dw | dry weight  
FAIM | Food Additive Intake Model  
FAO | Food and Agriculture Organization  
FDA | Food and Drug Administration  
GMP | Good Manufacturing Practice  
GRAS | Generally Recognized As Safe  
HACCP | Hazard Analysis Critical Control Points  
HAU | Haemagglutination Units  
ICP-MS | inductively coupled plasma mass spectrometry  
LC-MS/MS | liquid chromatography with tandem mass spectrometry  
LOD | limit of detection  
NDA | EFSA Panel on Nutrition, Novel Foods and Food Allergens  
NOAEL | no observed adverse effect level  
NF | novel food  
PDCAAS | Protein Digestibility Corrected Amino Acid Score  
SD | standard deviation  
SDS-PAGE | sodium dodecyl sulfate-polyacrylamide gel electrophoresis  
TIU | Trypsin Inhibitor Units  
UCT | University of Chemistry and Technology (Prague)  
UNU | United Nations University  
USDA | United States Department of Agriculture  
WHO | World Health Organization  
w/w | weight per weight
### Appendix A – Amino acid composition of the NF

Amino acid profile of mung bean protein (mg amino acid/100 g NF). Values for hydroxyproline of batch#1 are given in the CoA in mg/g NF.

| Amino acid    | Analytical method | Batches | Mean [mg/100 g] | SD [mg/100 g] |
|---------------|-------------------|---------|-----------------|---------------|
|               |                   | #1      | #2              | #3            | #4            |
| Aspartic acid | Covance TAALC_S:17| 9,770   | 9,900           | 9,770         | 9,848         |
| Threonine     |                   | 2,220   | 2,300           | 2,230         | 2,239         |
| Serine        |                   | 4,210   | 4,230           | 4,280         | 4,231         |
| Glutamic acid |                   | 14,800  | 14,500          | 14,600        | 14,667        |
| Glycine       |                   | 2,660   | 2,750           | 2,660         | 2,683         |
| Alanine       |                   | 3,140   | 3,160           | 3,110         | 3,077         |
| Valine        |                   | 4,350   | 4,420           | 4,280         | 4,367         |
| Methionine    |                   | 1,030   | 1,050           | 1,020         | 1,030         |
| Isoleucine    |                   | 3,820   | 3,930           | 3,790         | 3,876         |
| Leucine       |                   | 6,760   | 6,950           | 6,810         | 6,849         |
| Tyrosine      |                   | 2,550   | 2,660           | 2,570         | 2,592         |
| Phenylalanine |                   | 5,370   | 5,600           | 5,530         | 5,506         |
| Lysine        |                   | 5,490   | 5,690           | 5,690         | 5,643         |
| Histidine     |                   | 2,280   | 2,340           | 2,290         | 2,289         |
| Arginine      |                   | 5,840   | 5,960           | 5,940         | 5,922         |
| Amino acid   | Analytical method | Batches | Mean [mg/100 g] | SD [mg/100 g] |
|-------------|-------------------|---------|----------------|--------------|
|             |                   | #1 | #2 | #3 | #4 |                  |            |
| Proline     |                   | 3,460 | 3,590 | 3,610 | 3,530 | 3,541 | 68 |
|             |                   | 3,420 | 3,600 | 3,600 | 3,470 |            |            |
|             |                   | 3,470 | 3,560 | 3,610 | 3,570 |            |            |
| Hydroxyproline |                 | 28.3 | 26.7 | 27.4 | 27.5 | 28 | 1 |
|             |                   | 29.9 | 25.9 | 28.1 | 27.1 |            |            |
|             |                   | 29.1 | 26.9 | 27.4 | 28.2 |            |            |
| Cystine     |                   | 263 | 261 | 300 | 274 | 271 | 23 |
|             |                   | 243 | 251 | 309 | 251 |            |            |
|             |                   | 264 | 258 | 310 | 267 |            |            |
| Tryptophan  | Covance TRPLC_S:13 | 735 | 739 | 789 | 767 | 761 | 27 |
|             |                   | 734 | 740 | 792 | 770 |            |            |
|             |                   | —   | 734 | 806 | 770 |            |            |
Appendix B – Batch analysis of antinutritional factors, phenolics and cyanogenic glycosides in the mung bean flour

| Parameter (Unit)                          | Batches                          | Method of analysis                                           |
|------------------------------------------|----------------------------------|--------------------------------------------------------------|
| Lectin (HAU/g)                           | < 120 < 120 < 120 < 120 < 120   | Lectin testing by haemagglutination (TES-AC358)              |
| Phytic acid (mg/g)                       | 6.59 6.93 6.27 6.42 6.54         | Phytic acid (PHYT_S)                                         |
| Trypsin inhibitors (TIU/mg)              | 4.01 6.50 3.82 3.57 4.21         | Trypsin inhibitor (TRYP_IN_S)                                |
| Tannins                                  | 1.26 1.41 1.32 1.08 1.28         | Folin Ciocalteu spectrophotometric assay (gallic acid equivalents) |
|                                          | 7.63 14.35 9.37 15.63 13.60      | DMAC spectrophotometric assay (procyanidin A2 equivalents)   |
| Cyanogenic glycosides (µg/g)             | < LOD < LOD < LOD < LOD < LOD   | LC-MS/MS                                                    |

HAU: Haemagglutination Units; TIU: Trypsin Inhibitor Units; DMAC: dimethylaminocinnamaldehyde; LOD: limit of detection of 5 ppb; SD: standard deviation.
Appendix C – Magnesium and sodium minerals intake from the NF

### C.1. Intake estimates of magnesium (mg/day) resulting from the use of the NF as an ingredient in the intended food categories at the maximum proposed use levels

| Population group       | Max average | Max 95th |
|------------------------|-------------|----------|
| Infants                | 0.3         | 0        |
| Young children         | 6.7         | 0.7      |
| Other children         | 4           | 17.1     |
| Adolescents            | 0.9         | 4.3      |
| Adults                 | 2.2         | 18.7     |
| Elderly and very elderly| 1.4         | 5        |

### C.2. Intake estimates of sodium (mg/day) resulting from the use of the NF as an ingredient in the intended food categories at the maximum proposed use levels

| Population group       | Max. average | Max. 95th |
|------------------------|--------------|-----------|
| Infants                | 3.6          | 0         |
| Young children         | 84.1         | 9.8       |
| Other children         | 50           | 213.5     |
| Adolescents            | 12.2         | 53.6      |
| Adults                 | 28           | 234.2     |
| Elderly and very elderly| 18.3        | 62.2      |
Annex A – Dietary exposure estimates to the Novel Food for each population group from each EU dietary survey as derived from the FAIM tool

Information provided in this Annex is shown in an Excel file (downloadable at https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2021.6846#support-information-section).