Safety of frozen and dried formulations from migratory locust (Locusta migratoria) as a Novel food pursuant to Regulation (EU) 2015/2283

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Safety of frozen and dried formulations from migratory locust (*Locusta migratoria*) 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 Food and Food Allergens (NDA) was asked to deliver an opinion on the safety of frozen and dried formulations from migratory locust (Locusta migratoria) as a novel food pursuant to Regulation (EU) 2015/2283. The term migratory locust refers to the adult of the insect species Locusta migratoria. The NF is proposed in three formulations i) frozen without legs and wings; ii) dried without legs and wings; iii) ground with legs and wings. The main components of the NF are protein, fat and fibre (chitin) in the dried form of the NF, and water, protein, fat and fibre (chitin) in the frozen form of the NF. The Panel notes that the concentration of contaminants in the NF depends on the occurrence levels of these substances in the insect feed. The Panel notes that there are no safety concerns regarding the stability of the NF if the NF complies with the proposed specification limits during its entire shelf-life. The NF has a high protein content, although the true protein levels in the NF are overestimated when using the nitrogen-to-protein conversion factor of 6.25, due to the presence of non-protein nitrogen from chitin. The applicant proposed to use the NF as frozen, dried and ground in the form of snack, and as a food ingredient in a number of food products. The target population proposed by the applicant is the general population. The Panel notes that considering the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous. The submitted history of use and toxicity studies from literature did not raise safety concerns. The Panel considers that the consumption of the NF might trigger primary sensitisation to *L. migratoria* proteins and may cause allergic reactions in subjects with allergy to crustaceans, mites and molluscs. Additionally, allergens from the feed may end up in the NF. The Panel concludes that the NF is safe under the proposed uses and use levels.

Keywords: Novel food, food safety, *Locusta migratoria*, migratory locust, edible insects

Requestor: European Commission

Question number: EFSA-Q-2019-00115

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1. Introduction

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

On 28 December 2018, the company Fair Insects BV (A Protix Company) submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283 to authorize placing on the market of whole and ground grasshoppers (*Locusta migratoria*) as a novel food.

The target population is general population, excluding infants and young children. The applicant has also requested data protection under Article 26 of Regulation (EU) 2015/2283.

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 the safety of whole and ground grasshoppers (*Locusta migratoria*) as a novel food.

The European Commission asks the European Food Safety Authority to evaluate and inform the Commission as to whether and if so, to what extent, the requirements of Article 26(2)(c) of Regulation (EU) 2015/2283 are fulfilled in elaborating its opinion on whole and ground grasshoppers (*Locusta migratoria*) regarding the proprietary data for which the applicant is requesting data protection.

In the process of the evaluation of this Novel Food, it became apparent that the Commission should amend the title of the mandate in relation to the terms "whole" and "grasshopper". The term "grasshopper" is generic and does not exclusively refer to *Locusta migratoria*, and the term "whole" is not accurate as some of the parts may need to be removed prior to its consumption. On that basis, the Commission amended the title to "Revised request for a scientific opinion on frozen and dried formulations from migratory locust (*Locusta migratoria*) as a novel food".

1.2. Interpretation of the Terms of Reference

Given the proposed intended uses and in accordance to Art 5 of the Commission Implementing Regulation (EU) 2017/2469 stating '*where it cannot be excluded that a novel food intended for a particular group of the population would be also consumed by other groups of the population, the safety data provided shall also cover those groups*', it was clarified that the target population is the general population.

The applicant was requested to provide a revised assessment for the anticipated intake considering all population groups.

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 EFSA's requests for supplementary information. 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 Dibusz and Vejvodova (2020).

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[1].

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of an 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 description of the production process, analytical data on the composition of the NF, analytical data on contaminants in the NF, stability and microbiological status, data on NF sales, intake assessment, protein digestibility and DIAAS, genotoxicity and cytotoxicity study.

[1] 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.

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.

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 NF subject of the application is formulations of *Locusta migratoria* sp. (migratory locust), an insect species that belongs to the family of Acrididae. The NF falls under the category 'food consisting of, isolated from or produced from animals or their parts', as described in Article 3 of Regulation (EU) 2015/2283. The NF is produced by farming and processing of *L. migratoria* and consists mainly of protein, fat and fibre (dry basis). The NF is proposed to be marketed as frozen, dried or in the form of powder. The applicant proposes to use the NF as ingredient in various food products such as breakfast cereals, pasta, bakery products, sauces, meat products and meat imitates. Products with the NF can be consumed by the general population.

According to Regulation (EU) 2015/2283, this NF falls under the following category:

i) food consisting of, isolated from or produced from animals or their parts, except for animals obtained by traditional breeding practices which have been used for food production within the Union before 15 May 1997 and the food from those animals has a history of safe food use within the Union.

3.2. Identity of the NF

The NF consists of frozen, dried and ground formulations of *Locusta migratoria* sp. (migratory locust). The term 'migratory locust' refers to the adult of *Locusta migratoria*, an insect species that belongs to the Family of Acrididae, Subfamily Locustinae, Genus Locusta. The following scientific synonyms have been described in Global Biodiversity Information Facility – (GBIF Secretariat, 2019), Gryllus (*Locusta*) *migratorius* Linnaeus 1758; *Pachytylus migratorius*, Rehn 1902; *Gryllus (Locusta) danicus*, Linnaeus 1767; *Locusta danica*, Ikonnikov 1913; *Pachytylus danicus*, Doi 1932; *Locusta migratoria* Danica, Ju 1969; *Gryllus (Locusta) cinerascens Fabricius*, 1781; *Pachytylus cinerascens*, Walker 1870.

L. migratoria sp. is currently present in various regions worldwide, including Australia, Asia, Africa and Europe (GBIF Secretariat, 2019). The identity of the insect species has been certified by means of morphological identification by the applicant in collaboration with certified taxonomist in the Netherlands. Due to density-dependent phase polyphenism, *L. migratoria* exists as two phenotypes, solitary and gregarious. The applicant ensures that adults are reared and harvested during solitary phase by controlling rearing conditions.

The NF is intended to be marketed as A) blanched and frozen *L. migratoria* (LM frozen); B) blanched and freeze-dried *L. migratoria* (LM dried); C) blanched, freeze-dried and ground *L. migratoria* (LM powder). The insects are farmed under controlled rearing conditions.

3.3. Production process

According to the information provided, the NF is produced in line with Hazard Analysis Critical Control Points (HACCP) principles. The applicant stated that insects were reared at a facility registered at the Netherlands Food and Consumer Product Safety Authority (NVWA) as food-producing company. The production process can be divided into three distinct phases, i.e. farming, harvest and post-harvest processing. All steps take place under controlled rearing conditions.

Farming includes mating of the adult insect population and rearing of the nymphs. The eggs are separated from the adult insects so that nymphs can consequently grow separately. After being hatched from the eggs, the nymphs grow under monitored temperature and humidity conditions, in stainless steel

containers, certified for food contact and regularly disinfected. The applicant reported that no antimicrobials, veterinary medicinal products or solvents are used during the entire production process.

The applicant reported that the feed used to feed *L. migratoria* is a plant-derived material compliant with Directive 2002/32/EC[2] and produced according to Good Manufacturing Practice (GMP+).

During farming, *L. migratoria* can be infected by pathogens, including bacteria, viruses, entomopathogenic fungi, microsporidia and protists (Eilenberg et al., 2015). Pathogens that may affect *L. migratoria* include the virus Cricket iridovirus (CrIV) (Kleespies et al., 1999), the fungus *Metarhizium acridum* (Eilenberg et al., 2015) and a microsporidium named *Paranosema locustae* (Maniania et al., 2008). Literature review conducted by the applicant highlighted that these pathogens are specific at species or family level, and non-pathogenic for humans or other vertebrates. An example of food-borne bacteria to which *L. migratoria* is sensitive is *Bacillus thuringiensis* (Song et al., 2008). However, its potential presence in the NF is monitored by microbiological analysis of *Bacillus* as reported in Section 3.4 Table 4. In fact, *B. cereus* and *B. thuringiensis* are considered indistinguishable in the context of clinical diagnostics and food microbiology (EFSA BIOHAZ Panel, 2016).

Adults are harvested (3–5 weeks old) after being separated from the substrate and faeces. Decayed insects are identified by visual inspection and removed from the rearing batches. After the harvest, a minimum of 24 h fasting step is implemented to allow the adults to discard their bowel contents.

The post-harvesting processing includes killing of the adults by freezing and storing at –18°C. Three formulations of the NF are then obtained by processing. The formulation 'LM frozen' is obtained after removing of legs and wings, rinsing in water, blanching in hot water (> 90°C for at least 10 min) and freezing. The formulation 'LM dried' is obtained after removing legs and wings, rinsing in water, blanching in hot water (> 90°C for at least 10 min) and freeze-drying. The formulation 'LM powder' is obtained after rinsing in water, blanching in hot water (> 90°C for at least 10 min), freeze-drying and grinding (including legs and wings).

The thermal treatment contributes to the reduction of the microbial load of the NF as well as the elimination of potentially present viruses, parasites and reduction of enzymatic activity. Dehydration of the insects takes place in freeze dryers, resulting in a final product with moisture < 5%. Body parts (i.e. legs and wings) are removed by the manufacturer (LM frozen and LM dried) to reduce the risk of intestinal constipation that could be possibly caused by ingestion of the large spines on the insect tibia (FAO, 2013a). The NF LM powder is obtained via mechanical grinding of the insect (with legs and wings) and sieving to reduce particle size below 1 mm. The formulations of the NF are packed in hermetically closed packaging and stored at –18°C (LM frozen), or at room temperature (LM dried, LM powder).

The Panel considers that the production process is sufficiently described.

3.4. Compositional data

In order to confirm that the manufacturing process is consistent and adequate to produce on a commercial scale a product with certain characteristics, the applicant provided qualitative and quantitative data on chemical and microbiological parameters for a number of different batches of the NF formulations i.e. a) LM frozen; b) LM dried; c) LM powder. For all parameters, at least five independently produced batches were analysed. Considering the production process, the Panel accepted the view that the two formulations of the NF (LM frozen and LM dried) are representative of each other regarding the compositional parameters, when the difference in moisture is taken into account. Where indicated, analyses were performed on LM dried including legs and wings and considered as representative of LM powder. Microbiological analyses were performed on all formulations of the NF.

Certificates of accreditation of the laboratories that conducted the analyses were provided by the applicant. Analytical data were produced using methods validated for other types of matrices. Whenever in-house methods were employed, a full description of the method as well as results of the respective validation procedures have been provided.

The NF is a 'whole food' as defined by EFSA Scientific Committee (2011), meaning that all its constituents cannot be fully identified and/or characterised (EFSA NDA Panel, 2016).

The results of the proximate analysis of the NF are presented in Table 1. The amino acid, fatty acid, vitamin and mineral composition are reported in Section 3.9.

[2] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002, p. 10.

Table 1: Batch to batch analysis of the NF (LM frozen*, LM dried, LM powder)

LM frozen	LMFNFD01	LMFNFD02	LMFNFD03	LMFNFD04	LMFNFD05	Analytical method
Parameter (unit)						
Crude protein (g/100 g of NF)	14.5	14.5	14.5	14.3	14.3	Dumas, (N × 6.25)
Fat (g/100 g of NF)	11.3	12.0	11.9	10.9	10.3	Gravimetry EC-152/2009
Digestible carbohydrates (g/100 g of NF)	0.2	0.2	0.2	0.2	0.3	Titrimetry (Luff Schoorl)
Dietary fibre (g/100 g of NF)	2.6	2.6	2.5	2.7	2.5	EC-152/2009
Sugars (g/100 g of NF)**	–	–	–	–	–	NEN-3571; EC-152/2009
Ash (g/100 g of NF)	0.7	0.7	0.9	0.7	0.9	EC-152/2009
Moisture (g/100 g of NF)	71.5	71.5	71.5	71.5	71.5	Gravimetric method
Energy value (kJ/100 g of NF)	672	717	722	674	660	Regulation (EU) 1169/ 2011
Energy value (kcal/100 g of NF)	161	171	173	161	158	Regulation (EU) 1169/ 2011
LM dried						
Parameter (unit)	**LMDNFD01**	**LMDNFD02**	**LMDNFD03**	**LMDNFD04**	**LMDNFD05**	
Crude protein (g/100 g of NF)	48.7	48.8	48.9	48.1	48.3	Dumas, (N × 6.25)
Fat (g/100 g of NF)	38.1	40.4	40.1	36.6	34.8	Gravimetry EC-152/2009
Digestible carbohydrates (g/100 g of NF)	0.8	0.8	0.8	0.8	0.9	Titrimetry (Luff Schoorl)
Dietary fibre (g/100 g of NF)	8.8	8.8	8.3	9.0	8.6	EC-152/2009
Sugars (g/100 g of NF)	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6	NEN-3571; EC-152/2009
Ash (g/100 g of NF)	2.3	2.2	3.1	2.5	3.0	EC-152/2009
Moisture (g/100 g of NF)	4.2	4.3	4.3	4.2	4.4	Gravimetric method
Energy value (kJ/100 g of NF)	2,263	2,416	2,433	2,270	2,222	Regulation (EU) 1169/ 2011
Energy value (kcal/100 g of NF)	541	577	581	543	531	Regulation (EU) 1169/ 2011
LM powder						
Parameter (unit)	**LMGNFD01**	**LMGNFD02**	**LMGNFD03**	**LMGNFD04**	**LMGNFD05**	
Crude protein (g/100 g of NF)	55.7	55.6	57.2	52.5	53.8	Kjeldahl (N × 6.25)
Fat (g/100 g of NF)	35.8	34.2	33.0	38.5	36.8	Gravimetric method

LM frozen	LMFNFD01	LMFNFD02	LMFNFD03	LMFNFD04	LMFNFD05	Analytical method
Digestible carbohydrates (g/100 g of NF)	2.4	2.4	2.0	1.7	1.9	Titrimetry (Luff Schoorl)
Dietary fibre (g/100 g of NF)	7.4	7.6	7.0	6.5	6.6	AOAC 2009.01
Sugars** **(g/100 g of NF)**	0.24	0.22	0.23	0.19	0.20	HPAEC-PAD
Ash (g/100 g of NF)	1.9	1.9	1.9	1.9	1.9	Gravimetric method
Moisture (g/100 g of NF)	1.2	1.1	1.2	2.4	1.0	Gravimetric method
Energy value (kJ/100 g of NF)	2,400	2,300	2,300	2,400	2,400	Regulation (EU) 1169/ 2011
Energy value (kcal/100 g of NF)	570	550	550	570	560	Regulation (EU) 1169/ 2011

*: Derived by calculation by the applicant from LM dried by considering moisture.
**: Glucose, fructose, lactose, sucrose, maltose; AOAC: Association of Official Agricultural Chemists; HPAEC-PAD: High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection.

The Panel notes that there is a variation of the values of some proximate parameters, but this can be expected due to analytical variation and since the NF is produced using whole insects. The compositional values may also depend on the rearing conditions (feed, developmental stage at the time of harvesting, ambient conditions (Oonincx and van der Poel, 2011; Rumpold and Schlüter, 2013a).

Regarding the crude protein content of the NF, the Panel notes that Janssen et al. (2017) suggest that it is possibly overestimated when using the nitrogen-to-protein conversion factor of 6.25, mainly due to the presence of chitin. This issue will be addressed in detail in Section 3.9.

Chitin is the main form of dietary fibre in *L. migratoria* (Oonincx and van der Poel, 2011; Hahn et al., 2018). It is a linear polysaccharide constituted by β-(1,4)-linked 2-amino-2-deoxy-β-D-glucopyranose and 2-acetamido-2-deoxy-β-D-glucopyranose residues (Roberts, 1992). Due to the differences in body parts, after EFSA's request, the applicant provided analytical data on the levels of chitin in 5 independently produced batches in two formulations of the NF (LM dried and LM powder). The panel notes that a nationally or internationally recognised reference method for the analytical determination of chitin does not exist. The chitin content in the NF was based on the protocol described by Hahn et al. (2018), in which chemical treatment based on Acid Detergent Fibre – Acid Detergent Lignin is used to estimate the chitin content in different insects. The Panel considers that the differences between the content of dietary fibre (Table 1) and chitin (Table 2) are due to different analytical methods utilised.

Table 2: Chitin content of the Novel Food formulations (LM frozen*, LM dried, LM powder)

LM frozen					
Chitin (g/100 g NF)	LM frozen 01	LM frozen02	LM frozen03	LM frozen04	LM frozen05
	1.77	1.74	1.77	1.80	1.77
LM dried					
Chitin (g/100 g NF)	LM dried01	LM dried02	LM dried03	LM dried04	LM dried05
	6.5	6.4	6.5	6.6	6.5
LM powder					
Chitin (g/100 g NF)	LM powder01	LM powder02	LM powder03	LM powder04	LM powder05
	12.0	12.1	12.1	10.5	11.9

*: Derived by calculation by the applicant from LM dried by considering moisture.

Levels of heavy metals in LM powder are reported in Table 3. The applicant compared the values to the maximum levels for other foods as set in Regulation (EC) No. 1881/2006. The Panel notes that the levels of heavy metals reported for the NF are comparable to those set for other foods, and that in the current EU legislation, no maximum levels of heavy metals are set for insects as food.

Analytical data on the concentrations of aflatoxins B1, B2, G1, G2, ochratoxin A, nivalenol, deoxynivalenol, zearalenone, T2- and HT2-toxins and, upon EFSA's request, fumonisin B1 and fumonisin B2, were provided (Table 3). Values were compared to maximum limits for different foods set in EC Regulation (EC) No 1881/2006. The Panel notes that, in the current EU legislation, no maximum levels of mycotoxins are set for insects as food.

The content of dioxins and dioxins-like compounds were provided by the applicant (Table 3) and values were compared to maximum levels for other foods as set in Regulation (EC) No 1881/2006). The Panel notes that in the current EU legislation, no maximum levels of dioxins-like compounds are set for insects as food.

Table 3: Heavy metals, mycotoxins and dioxins in LM powder*

Parameter	Analytical method	LM 01	LM 02	LM 03	LM 04	LM 05
Heavy metals (mg/kg)						
Arsenic (As)	Internal method, ICP-MS[a]	0.01	0.01	0.01	< 0.02	0.02
Mercury (Hg)		0.0018	0.0020	0.0012	0.0022	0.0018
Lead (Pb)		0.03	0.04	0.03	0.07	0.06
Cadmium (Cd)		0.04	0.05	0.04	0.05	0.04
Mycotoxins (μg/kg)						
Aflatoxins B1	Internal Method, IAC-LC-FLD[b]	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10
Aflatoxins B2		< 0.04	< 0.04	< 0.04	< 0.04	< 0.04
Aflatoxins G1		< 0.10	< 0.10	< 0.10	< 0.10	< 0.10
Aflatoxins G2		< 0.06	< 0.06	< 0.06	< 0.06	< 0.06
Aflatoxins (Sum of B1, B2, G1, G2)		< 0.30	< 0.30	< 0.30	< 0.30	< 0.30
Ochratoxin A	Internal Method, IAC-LC-FLD[b]	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4
Nivalenol	Internal Method, LC-MS/MS[c]	< 20	< 20	< 20	< 20	< 20
Deoxynivalenol		< 20	< 20	< 20	< 20	< 20
Zearalenone		< 10	< 10	< 10	< 10	< 10
T-2 and HT-2		< 20	< 20	< 20	< 20	< 20
Fumonisin B1	Internal adaptation of NEN-EN 17194:2017-o, LC-MS/MS	< 0.0073	< 0.0073	< 0.0073	< 0.0073	< 0.0073
Fumonisin B2		< 0.0031	< 0.0031	< 0.0031	< 0.0031	< 0.0031
Dioxins (pg/g fat)						
WHO (2005) PCDD/F+PCB[d] TEQ (upper bound)	EC 2017/644, GC-MS/MS[e]	1.0	1.0	1.1	1.3	1.0

*: Analyses performed on LM dried including legs and wings.
(a): ICP-MS = inductively coupled plasma-mass spectrometry.
(b): IAC-LC/FLD = immunoaffinity chromatography-liquid chromatography/fluorescence detector.
(c): LC-MS/MS = liquid chromatography/tandem mass spectrometry.
(d): WHO (2005) PCDD/F+PCB = sum of polychlorinated dibenzo-para-dioxins-polychlorinated dibenzofurans-polychlorinated biphenyls expressed as World Health Organization toxic equivalent.
(e): GC-MS/MS = gas chromatography/tandem mass spectrometry.

Analytical data of the pesticide concentrations for five independently produced batches of the NF have been provided. The results showed that the tested pesticide concentrations in the LM powder are below the limits of quantification (LOQ) of the implemented method used (GC-MS ITD Equal CEN/TR

16468 and LC-MS Equal CEN/TR 15641) and are complying with Regulation (EC) No 396/2005[3] defining maximum residue limits (MRL) of pesticides in foods.

Given the vegetable origin of the feeding substrate and the absence of prion or prion-related encoding genes in insects, the development of specific prion diseases due to the consumption of the NF is not expected (EFSA Scientific Committee, 2015).

The applicant provided analytical data for histamine for five independently produced batches of LM dried and LM powder (all below 10 mg/kg) and values were compared to the limit of 200 mg/kg for histamine in fishery products set in Commission Regulation EC No 2073/2005. High concentrations of putrescine (470–620 mg/kg in LM dried and 279–299 mg/kg in LM powder) were reported. No legal limit has been established for putrescine in any food although it may accumulate at very high concentration in cheese (up to 1,560 mg/kg), fermented sausages (up to 1,550 mg/kg) and fish sauces (up to 1,220 mg/kg) (EFSA BIOHAZ Panel, 2011). A recent study by del Rio et al. (2018) described a real-time analysis of the cytotoxicity of putrescine and cadaverine on intestinal cell cultures and found that the LOAEL (lowest observed adverse effect level) for putrescine was 881.50 mg/kg and for cadaverine 510.89 mg/kg. Formation of biogenic amines can occur by endogenous biosynthesis, uptake from the feed source and by bacteria of the intestinal microbiota of insects. It can also occur during food processing and storage as result of bacterial contamination (EFSA BIOHAZ Panel, 2011). Upon EFSA's request, the applicant was asked to analyse the NF for *Pseudomonas aeruginosa*, which could also be responsible for biogenic amines production. All formulations of the NF were tested and levels of < 10 cfu/g were reported.

The applicant provided microbiological data on five independently produced batches of all NF formulations (LM frozen, LM dried, LM powder) (Table 4).

The Panel notes that the applicant did not provide the actual values of the microbiological parameters, but instead the quantification limits as defined by the dilutions used upon the analyses. Furthermore, the Panel notes that the microbiological values of the analysed samples do not exceed the given specification limits.

Table 4: Microbiological analyses of the NF

LM frozen (without legs and wings)	**Batch**					
	Units	**LMPNFD01**	**LMPNFD02**	**LMPNFD03**	**LMPNFD04**	**LMPNFD05**
Total aerobic count	(cfu/g)	< 1,000	< 1,000	< 1,000	< 1,000	< 1,000
Enterobacteriaceae	(cfu/g)	< 10	< 10	< 10	< 10	< 10
Escherichia coli	(cfu/g)	< 10	< 10	< 10	< 10	< 10
Listeria monocytogenes	In 25 g	ND	ND	ND	ND	ND
Salmonella	In 25 g	ND	ND	ND	ND	ND
Bacillus cereus (spores)	(cfu/g)	< 10	< 10	< 10	< 10	< 10
Coagulase positive staphylococci	(cfu/g)	< 10	< 10	< 10	< 10	< 10
Campylobacter **spp.**	(cfu/g)	ND	ND	ND	ND	ND
Clostridium perfringens	In 25 g	< 10	< 10	< 10	< 10	< 10
Yeasts and moulds	(cfu/g)	< 40	< 10	< 10	< 10	< 10
LM dried (without legs and wings)	**Batch**					
	Units	**LMDNFD01**	**LMDNFD02**	**LMDNFD03**	**LMDNFD04**	**LMDNFD05**
Total aerobic count	(cfu/g)	< 1,000	< 1,000	< 1,000	< 1,000	< 1,000
Enterobacteriaceae	(cfu/g)	< 10	< 10	< 10	< 10	< 10
Escherichia coli	(cfu/g)	< 10	< 10	< 10	< 10	< 10
Listeria monocytogenes	In 25 g	ND	ND	ND	ND	ND
Salmonella	In 25 g	ND	ND	ND	ND	ND
Bacillus cereus (spores)	(cfu/g)	< 10	< 10	< 10	< 10	< 10

[3] Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. OJ L 70, 16.3.2005, p. 1.

Coagulase positive staphylococci	(cfu/g)	< 10	< 10	< 10	< 10	< 10
Campylobacter **spp.**	(cfu/g)	ND	ND	ND	ND	ND
Clostridium perfringens	(cfu/g)	< 10	< 10	< 10	< 10	< 10
Yeasts and moulds	(cfu/g)	< 40	< 10	< 10	< 10	< 10
LM powder (with legs and wings)		**Batch**				
	Units	**LMPNFD01-0M**	**LMPNFD02-0M**	**LMPNFD03-0M**	**LMPNFD04-0M**	**LMPNFD05-0M**
Total aerobic count	(cfu/g)	< 40	< 40	< 10	< 10	< 40
Enterobacteriaceae	(cfu/g)	< 10	< 10	< 10	< 10	< 10
Escherichia coli	(cfu/g)	< 10	< 10	< 10	< 10	< 10
Listeria monocytogenes	In 25 g	ND	ND	ND	ND	ND
Salmonella	In 25 g	ND	ND	ND	ND	ND
Bacillus cereus (spores)	(cfu/g)	< 10	< 10	< 10	< 10	< 10
Coagulase positive staphylococci	(cfu/g)	< 10	< 10	< 10	< 10	< 10
Campylobacter **spp.**	(cfu/g)	ND	ND	ND	ND	ND
Clostridium perfringens	(cfu/g)	< 10	< 10	< 10	< 10	< 10
Yeasts and moulds	(cfu/g)	< 10	< 10	< 10	< 10	< 10

cfu: colony forming units; ND: not detected.

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

3.4.1. Stability

The applicant provided data on the microbiological profile of five batches of the novel food (LM frozen, LM dried), and upon request (LM powder). The applicant proposed a shelf-life of 12 months for each formulation. The NF formulations have been analysed immediately after manufacturing (0 months) and after storage at room temperature (LM dried and LM powder) or –18°C (LM frozen) for 12 months. Microbiological data at 3, 6 and 9 months were also provided for LM frozen and LM dried, and results were within acceptable values between 0 and 12 months. Microbiological data at 6 months were provided for LM powder, falling within 0–12 months values. The Panel notes that the microbiological values do not exceed the given specification limits.

Table 5: Microbiological status of the NF formulations during the proposed shelf-life

LM frozen (without legs and wings)	**Units**	**0 months**					**12 months**				
		LMPNFD01	**LMPNFD02**	**LMPNFD03**	**LMPNFD04**	**LMPNFD05**	**LMPNFD06**	**LMPNFD07**	**LMPNFD08**	**LMPNFD09**	**LMPNFD10**
Total aerobic count	(cfu/g)	< 1,000	< 1,000	< 1,000	< 1,000	< 1,000	< 4,000	< 1,000	< 2,000	< 1,000	< 1,000
Enterobacteriaceae	(cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Escherichia coli	(cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Listeria monocytogenes	In 25 g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella	In 25 g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bacillus cereus (spores)	(cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Coagulase positive Staphylococci	(cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Clostridium perfringens	(cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Yeasts and moulds	(cfu/g)	< 40	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10

LM dried (without legs and wings)	Units	0 months					12 months				
		LMDN FD01	LMDN FD02	LMDN FD03	LMDN FD04	LMDN FD05	LMDN FD06	LMDN FD07	LMDN FD08	LMDN FD09	LMDN FD10
Total aerobic count	(cfu/g)	< 1,000	< 1,000	< 1,000	< 1,000	< 1,000	< 10	< 10	< 10	< 10	< 10
Enterobacteriaceae	(cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Escherichia coli	(cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Listeria monocytogenes	In 25 g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella	In 25 g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bacillus cereus (spores)	(cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Coagulase positive Staphylococci	(cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Clostridium perfringens	(cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Yeasts and moulds	(cfu/g)	< 40	< 10	< 10	< 10	< 10	< 40	< 10	< 10	< 10	< 10
LM powder (with legs and wings)	**Units**	**0 months**					**12 months**				
		LMPN FD01	LMPN FD02	LMPN FD03	LMPN FD04	LMPN FD05	LMPN FD01	LMPN FD02	LMPN FD03	LMPN FD04	LMPN FD05
Total aerobic count	(cfu/g)	< 40	< 40	< 10	< 10	< 40	< 10	< 40	< 40	< 10	< 10
Enterobacteriaceae	(cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Escherichia coli	(cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Listeria monocytogenes	In 25 g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella	In 25 g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bacillus cereus (spores)	(cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Coagulase positive Staphylococci	(cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Clostridium perfringens	(cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Yeasts and moulds	(cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10

cfu: colony forming units; ND: not detected.

After EFSA's request, the applicant provided analytical data on the water activity and the oxidative status of the fats in the NF (LM powder) at 6 months (data not shown) and 12 months of shelf-life (Table 6). Peroxide value (PV), p-anisidine value (PA) and free fatty acids (FFA) have been determined. Regarding the relatively high p-anisidine value and its variability among batches (1.4–16.4) at t = 0 when compared to data at t = 12 months, the applicant indicated that such variation may be due to improper handling, packaging and storing of certain packages. The Panel notes that the variation was only observed in two batches.

Table 6: Water activity and oxidative status of fat in LM powder during the proposed shelf-life

LM powder (with legs and wings)	Units	0 months					12 months				
		LMPN FD01	LMPN FD02	LMPN FD03	LMPN FD04	LMPN FD 05	LMPN FD01	LMPN FD02	LMPN FD03	LMPN FD04	LMPN FD05
Water activity		0.382	0.384	0.376	0.377	0.379	0.377	0.575	0.572	0.510	0.429
Free fatty acids	% oleic acid	1.9	1.8	2.1	2.2	2.0	1.5	1.9	2.9	2.9	1.6
Peroxide value	Meq O_2/kg fat[(a)]	2.4	5.6	2.6	1.0	0.8	1.2	1.3	3.9	2.7	< 0.1
p-anisidine value		16.4	1.8	1.4	16.1	2.2	1.0	3.7	1.3	< 1.0	1.2

(a): Meq: milliequivalents.

Stability in the intended-for-use matrices. Since the NF is going to be used as an ingredient for the manufacturing of other foods, EFSA asked the applicant to investigate its stability when used as an ingredient in the intended-for-use matrices (see Section 3.7.2 Proposed uses and use levels). In particular, the applicant addressed the lipid hydrolysis and oxidation (FFA, PV and PA), the formation of process contaminants (polycyclic aromatic hydrocarbons - PAH) and microbiological stability of a wet food matrix composed of thermally processed beans and vegetables with 15% w/w LM frozen. The shelf-life of the intended-for-use matrix was addressed at t = 0 and t = 12 months.

Free fatty acids were reported in the range of 0.1–2.2% of total fat and 1.3–2.3% of oleic acid in the product at t = 0 and t = 12 months. Peroxide values of the product were below 0.1 meq/kg fat at t = 0 and t = 12 months, with one batch at 12 months showing 0.6 meq/kg fat. P-anisidine values in the products ranged from 14.6–19.8 at t = 0 to 31.7–65.9 at t = 12 months, showing the presence of secondary oxidation products. After EFSA's request, the applicant provided the content of p-anisidine in a control sample at t = 0, being 1.9. Literature reports that highly processed foods (e.g. mayonnaise) show a direct relation with FFA, PV and PA, especially when high pressure is applied (Sethi et al., 2017). When comparing the level of p-anisidine of canned vegetables with LM powder at 0 months, it can be concluded that the food matrix had an impact on the PA value.

The content of polycyclic aromatic hydrocarbons in the tested food at t = 0 was below 2.0 μg/kg, hence below the tolerable limits of crustaceans, cephalopods, muscle meat of fish, oils and fats, as set in Regulation (EC) No 1881/2006.

After EFSA's request, the applicant provided analytical data on the oxidative status of fats (FFA, PV and PA) and microbiological stability of the NF in meat imitates (80% w/w insect inclusion in dry matter of meat imitates) at t = 0 and under accelerated conditions (ASL) (80°C for 72 h with a humidity of about 55–60%). A meat imitate was prepared by mixing LM dried with oats, onions, beet root, salt, eggs and spices. A control sample was prepared without insects. Further after mixing and processing, the meat imitate was precooked in sunflower oil before packaging and freezing. Free fatty acids were reported in the range of 0.9% and 1.0%, respectively, at t = 0 and at ASL, compared to 1.2% in the control sample. Peroxide values were less than 0.1 meq/kg in the meat imitate at t = 0, 2.1 meq/kg in the meat imitate at ASL, and 3.9 meq/kg in the control sample. Values of p-anisidine were comparable between meat imitate and control at t = 0 (32.3 and 36.9, respectively) whereas it increased to 69.7 for meat imitate under accelerated conditions. There is no health-based guidance value or legislative limit for pAV in foods and the parameter is primarily used for quality control.

The Panel considers that the stability of the NF in the intended-for-use-matrices studied is well characterised and does not raise safety concerns.

The Panel notes that the analytical data regarding the putative formation of contaminants due to the use of NF as an ingredient in the intended-for-use matrices are limited, and no conclusion can be drawn due to the absence of proper control samples. The Panel notes that the food items containing the NF have to comply with existing microbiological criteria for foods as set in Commission Regulation (EC) No 2073/2005 and benchmark levels of acrylamide in bakery products established by Regulation (EU) 2017/2158. The Panel could not fully conclude on the stability of the NF based on the submitted data. However, provided that the specifications are met also at the end of the shelf-life, and that products containing the NF are compliant with respective legislative limits on process contaminants, the stability data do not raise safety concerns during the studied 12 months.

3.5. Specifications

The specifications of the NF as proposed by the applicant are indicated in Table 7.

Table 7: Specifications of the NF

Description:
LM frozen: thermally processed, frozen *Locusta migratoria* (without legs and wings)
LM dried: thermally processed, dried *Locusta migratoria* (without legs and wings)
LM powder: thermally processed, dried, grinded *Locusta migratoria* (with legs and wings)

Harvesting phase: solitary (Density-dependent phase polyphenism)

Parameters	Unit	LM frozen	LM dried	LM powder
Moisture	% w/w	67–73	≤ 5	≤ 5
Crude protein (N × 6.25)	% w/w	11–21	43–53	50–60
Fat	% w/w	7–13	31–41	31–41
Saturated fatty acids	% fat	35–43	35–43	35–43
Digestible carbohydrates	% w/w	0.1–2.0	0.1–2.0	1.0–3.5
Dietary fibre	% w/w	1.5–3.5	5.5–9.0	5.5–9.0
Chitin	% w/w	< 2.5	< 11	< 14
Peroxide value	Meq O_2/kg fat	≤ 5	≤ 5	≤ 5
Heavy metals				
– Lead	mg/kg	≤ 0.07	≤ 0.07	≤ 0.07
– Cadmium	mg/kg	≤ 0.05	≤ 0.05	≤ 0.05
Mycotoxins				
Aflatoxins (Sum of B1, B2, G1, G2)	µg/kg	≤ 0.3	≤ 0.3	≤ 0.3
Deoxynivalenol	µg/kg	≤ 20	≤ 20	≤ 20
Ochratoxin A	µg/kg	≤ 0.4	≤ 0.4	≤ 0.4
Processing contaminants				
Sum of dioxins and dioxins-like PCBs UB (WHO_{2005} PCDD/F-PCB-TEQ)	pg/g fat	≤ 1.2	≤ 1.2	≤ 1.2
Microbiological				
Total aerobic colony count	cfu/g	≤ 10^5	≤ 10^5	≤ 10^5
Enterobacteriaceae (presumptive)	cfu/g	≤ 100	≤ 100	≤ 100
Escherichia coli	cfu/g	≤ 50	≤ 50	≤ 50
Listeria monocytogenes		Not detected in 25g	Not detected in 25g	Not detected in 25g
Salmonella spp.		Not detected in 25g	Not detected in 25g	Not detected in 25g
Bacillus cereus (presumptive)	cfu/g	≤ 100	≤ 100	≤ 100
Coagulase positive Staphylococci	cfu/g	≤ 100	≤ 100	≤ 100
Sulfite-reducing Anaerobes	cfu/g	≤ 30	≤ 30	≤ 30
Yeasts and moulds	cfu/g	≤ 100	≤ 100	≤ 100

ND = not detected (i.e. 0); ADF-ADL = acid detergent fibre–acid detergent lignin; ICP-MS = Inductively Coupled Plasma-Mass Spectrometry; IAC-LC/FLD = immunoaffinity chromatography-liquid chromatography/fluorescence detector; GC-MS/MS = gas chromatography/tandem mass spectrometry; LC-MS/MS = Liquid chromatography/tandem mass spectrometry; CFU = colony forming units; dietary fibre may not include chitin due to different analytical methods, UB = upper bound; WHO-PCDD/F-PCB-TEQ: sum of polychlorinated dibenzo-para-dioxins-polychlorinated dibenzofurans-polychlorinated biphenyls expressed as World Health Organization toxic equivalent.

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

L. migratoria either collected from the wild or reared in farms is consumed as part of the customary diet in several non-EU countries worldwide. Human consumption of *L. migratoria* has largely been

documented in Madagascar, Cameroun, Congo, Zimbabwe, Sudan, South Sudan, Papua New Guinea, Thailand, China and Morocco (Jongema, 2017). Species of locusts are considered the most consumed insect species in Central African Republic (Durst et al., 2010). *L. migratoria* is commonly consumed as snack, side dish and in cooking sauces. Their preparation includes frying, roasting, boiling and sun drying and legs and wings are removed before consumption.

Since 2012, several companies and specialised shops have been selling *L. migratoria* in the EU either as whole food or by adding it to other food products. Since the 1 May 2017, *L. migratoria* is among the insect species that can be legally introduced in the Swiss market as food, when commercially reared.

L. migratoria can be found in the Dutch market since 2016.

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

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

3.7.2. Proposed uses and use levels

The NF formulations (frozen, dried and powder) is proposed to be used as an ingredient in several food products. These food products are defined using the FoodEx2 hierarchy, and the maximum use levels are reported in Table 8. The applicant intends to use different formulations of the NF (frozen, dried, powder) separately in the respective food category, and not in combination.

Table 8: Food categories and maximum use levels intended by the applicant

FoodEx2 level	FoodEx2 code	Food category	Max use levels (g NF/100 g)		
			LM frozen	LM dried	LM powder
L4	A03VD	Potato-based dishes	15	5	5
L4	A03VM	Legume-based dishes	15	5	5
L4	A03ZN	Pizza and pizza-like dishes	15	5	5
L3	A03TE	Meat imitates	80	50	50
L4	A0B9X	Tomato soup (dry)	20	5	5
L4	A0B9S	Mushroom soup (dry)	15	5	5
L4	A0B9R	Mixed vegetable soup (dry)	15	5	5
L4	A041P	Potato soup	15	5	5
L4	A041M	Onion soup	15	5	5
L4	A041Q	Legume (beans) soup	15	5	5
L4	A041N	Tomato soup	15	5	5
L4	A041R	Mushroom soup	15	5	5
L5	A041S	Mixed vegetable soup	15	5	5
L3	A01AZ	Canned or jarred legumes	20	15	15
L3	A0ETQ	Canned/jarred vegetables	20	15	15
L4	A042E	Caesar salad	15	5	5
L4	A042H	Prepared pasta salad	15	5	5
L2	A03MA	Beer and beer-like beverage	2	2	2
L2	A03PM	Mixed alcoholic drinks	2	2	2
L2	A04QF	Unsweetened spirits and liqueurs	2	2	2
L4	A0EQD	Chocolate and similar	30	10	10
L3	A06HL	Snacks other than chips and similar	100	100	100
L3	A01BJ	Primary derivatives from nuts and similar seeds	40	20	20
L5	A0BAV	Chickpeas (without pods)	40	20	20

FoodEx2 level	FoodEx2 code	Food category	Max use levels (g NF/100 g)		
			LM frozen	LM dried	LM powder
L3	A014C	Tree nuts	40	20	20
L3	A015F	Oilseeds	40	20	20
L4	A02QC	Frozen yoghurt	15	5	5
L3	A024F	Sausages	30	10	10

3.7.3. Anticipated intake of the NF

EFSA performed an intake assessment of the anticipated daily intake of the NF based on the applicant's proposed uses and maximum use levels (Table 8), using individual data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011). The lowest and highest mean and 95th percentile anticipated daily intake of the NF on a mg/kg body weight (bw) basis, among the EU dietary surveys are presented in Table 9.

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 9: Intake estimates resulting from the use of the NF as an ingredient in the intended food categories at the maximum proposed use levels

Population group	Age (years)	Mean intake (mg/kg bw per day)		P95th intake (mg/kg bw per day)	
		Lowest[(a)]	Highest[(a)]	Lowest[(b)]	Highest[(b)]
Infants	< 1	0	110	0	316
Young children[(d)]	1–< 3	27	524	176	1,370
Other children	3–< 10	65	356	244	977
Adolescents	10–< 18	22	226	105	671
Adults[(c)]	≥ 18	67	197	235	639

bw: body weight.
(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 24/03/2021. The lowest and the highest averages observed among all EU surveys are reported in these columns.
(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 24/03/2021. The lowest and the highest P95th observed among all EU surveys are reported in these columns (P95th based on less than 60 individuals are not considered).
(c): Includes elderly, very elderly, pregnant and lactating women.
(d): Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).

3.7.4. Estimate of exposure to undesirable substances

Based on the highest P95 intake estimate (Table 9), EFSA calculated the exposure to undesirable substances (heavy metals, toxins) for all population groups. The specification limits (Table 7) were used as maximum concentrations of the undesirable substances. When specification limits for a substance of possible concern have not been proposed, the maximum values reported for the analysed batches were used. Consumption of the NF under the proposed uses and use levels does not contribute substantially to the overall exposure to the analysed undesirable substances through diet.

The risk of intestinal constipation caused by the large spines on the tibia and chitinous material has been reported (FAO, 2013a) and can be reduced by removing legs and wings, or by reducing the particle size.

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

Not relevant.

3.9. Nutritional information

The applicant provided nutritional analyses of the NF formulations which consist mainly of protein, fat, dietary fibre (mainly chitin) and inorganic matter (LM dried, LM powder); water, protein, fat, dietary fibre (mainly chitin) and inorganic matter (LM frozen). The energy value of the NF is on average 690, 2,250 and 2,350 kJ/100 g, respectively, for LM frozen, LM dried and LM powder (Table 1). Analytical data on the amino acid composition, the fatty acid content, minerals and vitamins

in the NF formulations have been provided for five batches of LM powder (LM dried with legs and wings).

The NF contains on average 14.4 g crude protein per 100 g LM frozen, 48.6 g crude protein per 100 g LM dried and 55.0 g crude protein per 100 g LM powder, calculated using a protein conversion factor of 6.25. The Panel notes that the use of the conventional factor overestimates the levels of true protein content in *L. migratoria* due to the presence of considerable amounts of non-protein nitrogen derived mainly from chitin (Boulos et al., 2020). A study provided by the applicant identified a nitrogen-to-protein conversion factor of 5.31 for LM powder (LM dried with legs and wings). Using this factor, the protein content of the NF is about 15% lower than considering a conversion factor of 6.25. For regulatory purposes for nutritional labelling, protein is defined as the total nitrogen measured by the Kjeldahl method multiplied by a nitrogen-to-protein conversion factor of 6.25 [Regulation (EU) No 1169/2011 on the provision of food information to consumers].

The applicant quantified the amino acids in five batches of the NF according to ISO 13903:2005 and EU 152/2009 (Appendix A). The applicant also analysed the amount of amino acids in g per 100 g protein of LM powder (LM dried with legs and wings). Results show that in the protein from LM powder, all essential amino acids including sulfur containing amino acids were present in quantities similar or higher than the recommended levels by FAO (2013b) (Appendix A). Furthermore, the applicant conducted a study of the true ileal protein digestibility during transit through the dynamic *in vitro* gastrointestinal model (tiny-TIM). Casein was used as a reference protein. The tests were conducted by an accredited laboratory in accordance with GLP. The nitrogen digestibility was expressed as a percentage of the total nitrogen intake, including non-protein nitrogen. As result, the true ileal nitrogen digestibility of LM powder (LM dried with legs and wings) was 55.4% ± 2.4%, compared to casein 75.3% ± 1.4%, indicating that proteins of *L. migratoria* are less bio-accessible than casein. Following the recommendation by FAO, 2013b, the protein quality was determined by the Digestible Indispensable Amino Acid Score (DIAAS), using the reference value of child, adolescent, adult group (3–10 years). The DIAAS value for LM powder corresponded to 70%, compared to casein with a DIAAS of 91%. Sulfur amino acids (methionine + cysteine) were the limiting amino acids.

The major fatty acids in LM powder (LM dried with legs and wings) are palmitic acid, stearic acid, oleic acid, linoleic acid and alpha linolenic acid (Appendix B). On average, saturated, monounsaturated fats and polyunsaturated fatty acids (linoleic and alpha-linolenic acid) constitute 38.2%, 39.9% and 21.9% of total fatty acids, respectively. Similar fatty acids profile for *L. migratoria* is reported in literature (Clarkson et al., 2018). The average trans fatty acid content is 0.4% fat.

The applicant provided analytical data on the levels of some minerals and vitamins (Table 10), and after EFSA's request, boron, molybdenum, iodine and selenium. LM powder (LM dried with legs and wings) contains riboflavin, niacin, pantothenic acid, biotin and cobalamin besides vitamin E and small amounts of thiamine, folic acid, retinol and vitamin D. It also contains P, Zn, Cu and Mn.

Considering the mean contents reported in Table 10, values reported in the specifications, and the estimated P95 of exposure to the NF, the Panel notes that none of the existing upper levels for the analysed micronutrients are expected to be exceeded, for any population groups.

Table 10: Content of micronutrients (minerals and vitamins) in the NF LM powder*

Parameter	Analytical method	LMDNFD01	LMDNFD02	LMDNFD03	LMDNFD04	LMDNFD05
Minerals (mg/100 g)						
Calcium	ICP-MS	30	31	31	29	31
Copper		3.7	3.7	3.8	3.4	3.8
Iodine**		0.02	0.02	0.02	0.03	0.02
Iron		4.1	4.4	4.2	4.7	4.6
Magnesium		54	54	55	53	55
Manganese		0.33	0.35	0.35	0.33	0.33
Phosphorous		450	450	420	450	460
Potassium		410	490	500	470	460
Selenium**		< 0.03	0.04	0.05	< 0.03	< 0.03
Sodium		82	86	91	110	98
Zinc		18	19	19	17	14
Boron**	ICP-OES	0.2	0.2	0.1	0.2	0.2
Molybdenum**		< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Vitamins						
Alpha-tocopherol (mg/100 g)	EN 12822:2014	3.29	3.33	3.05	2.81	2.96
Biotin (µg/100 g)	LST AB 266.1, 1995	37.9	37.8	30.8	50.1	34.8
Cholecalciferol (µg/100 g)	EN 12821:2009	< 0.25 (LOQ)	< 0.25 (LOQ)	< 0.25 (LOQ)	< 0.25 (LOQ)	< 0.25 (LOQ)
Cobalamin (µg/100 g)	AOAC 2008, 91 4	1.21	0.9	0.77	0.62	1.01
Folic acid (µg/100 g)	AOAC 2013.13	< 5 (LOQ)	< 5 (LOQ)	< 5 (LOQ)	< 5 (LOQ)	< 5 (LOQ)
Methyltetrahydrofolate (5-MTHF) (µg/100 g)	AOAC 2013.13	15.1	14.7	13	11.4	12
Niacin (mg/100 g)	EN 15652:2009	7.17	6.67	7.21	7.03	6.49
Pantothenic acid (mg/100 g)	AOAC 2012.16	2.27	2.16	2.32	2.23	2.08
Pyridoxine hydrochloride (mg/100 g)	EN 14164	0.11	0.47	0.1	0.1	0.1
Retinol (µg/100 g)	EN 12823-1 2014	63.9	61.5	71.7	59.6	56.9
Riboflavin (mg/100 g)	EN 14152:2003	1.34	1.39	1.42	1.32	1.2
Thiamin (mg/100 g)	EN 14122:2003	0.08	0.07	0.08	0.07	0.07
Thiamin HCL (mg/100 g)	EN 14122:2003	0.1	0.09	0.1	0.09	0.09

*: Analyses performed on LM dried including legs and wings.
**: Analyses performed on LM powder: LOQ: limit of quantification; ICP-MS: Inductively coupled plasma mass spectrometry; ICP-OES: Inductively coupled plasma atomic emission spectroscopy.

It has been reported that chitin can be partially digested in the human stomach by the acidic mammalian chitinase (AMCase) (Paoletti et al., 2009; Muzzarelli et al., 2012). However, Paoletti et al. (2009) suggested that reduction of chitin intake in western diets may have led to reduced expression of chitinase genes, thus resulting in loss of catalytic efficacy. The NF contains on average 1.8 g, 6.5 g and 11.7 g chitin in 100 g LM in frozen, dried and powder formulations, respectively (see Table 2). The Panel considers that chitin is an insoluble fibre that is not expected to be digested in the small intestine of humans to any significant degree. It is also rather resistant to microbial fermentation and therefore assumed to be excreted mainly unchanged. Additionally, the Panel notes that chitin can bind bivalent minerals (Franco et al., 2004; Anastopoulos et al., 2017) possibly affecting their bioavailability, as reported for dietary fibres in general (Baye et al., 2017).

Insects may contain antinutritional factors (ANFs) such as tannins, oxalates, phytate, hydrogen cyanide (Jonathan et al., 2012; Shantibala et al., 2014), thiaminases (Nishimune et al., 2000) and protease inhibitors (Eguchi, 1993). The applicant determined the concentrations of total polyphenols, tannins, oxalic acid, phytic acid, hydrogen cyanide and trypsin inhibitors in five independently produced batches of LM powder (LM dried with legs and wings) (Table 11). The reported values in the NF are comparable to the occurrence levels of these compounds in other foodstuffs (Rao and Prabhavathi, 1982; Gupta, 1987; Holmes and Kennedy, 2000; Schlemmer et al., 2009; EFSA CONTAM Panel, 2019).

Table 11: Batch to batch analysis of anti-nutritional factors in LM powder*

Parameter (unit)	Analytical method	LMDNFD01	LMDNFD02	LMDNFD03	LMDNFD04	LMDNFD05
Total polyphenols (%)	In house method	0.48	0.45	0.41	0.44	0.46
Tannin (%)	Folin Denis method	0.5	0.7	0.6	0.6	0.6
Oxalic acid (mg/kg)	HPLC, in house method	< 100***	< 100***	< 100***	< 100***	< 100***
Phytic acid (g/kg)	ANAL-10445	1.0	1.3	1.8	1.3	1.1
Hydrogen cyanide (mg/kg)	NEN-EN 16160	< 1.5*	< 1.5*	< 1.5*	< 1.5*	< 1.5*
Trypsin inhibition activity (mg/g)	NEN-EN-ISO 14902	< 0.5*	< 0.5*	< 0.5*	< 0.5*	< 0.5*

*: Analyses performed on LM dried including legs and wings; HPLC: high performance liquid chromatography.
***: Below detection limit.

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

3.10. Toxicological information

Some insect species secrete chemical substances with potentially toxic effects, as part of their defence mechanisms (Dzerefos et al., 2013; Rumpold and Schlüter, 2013b). However, regarding *L. migratoria* the production of such substances has not been reported in the literature.

Regarding the safety of chitin present in the NF, the applicant referred to EFSA's scientific opinion on the safety of 'chitin-glucan' as a Novel Food ingredient (EFSA NDA Panel, 2010). However, the Panel considers that the polymer chitin-glucan cannot be considered as representative of the chitin derived from *L. migratoria*.

Potential adverse health effects of chitin may be related to immunological effects. As reviewed by Komi et al. (2018), chitin has been shown to activate a variety of innate (eosinophils, macrophages) and adaptive immune cells (IL-4/IL-13 expressing T helper type-2 lymphocytes) and this implies the potential to promote immunological reactions including hypersensitivity. EFSA identified an article (Niho et al., 1999) (Japanese language, only abstract available in English) stating that no toxic effects related to chitin were observed in F344 rats at concentrations up to 5% of chitin in the diet for 13 weeks. No firm conclusion could be drawn by the Panel since only the abstract was accessible.

The applicant has taken commitment for two toxicity studies to be performed with the aqueous extract from NF. However, due to the insolubility of the test item only one study assessing cytotoxicity was performed.

In addition, the applicant provided a study retrieved from literature (Turkez et al., 2014) which investigates the *in vitro* genotoxic potential of water soluble extracts of *L. migratoria* on cultured human blood cells. Additional publications were also identified by EFSA describing subacute and subchronic toxicity studies performed in the rat with powdered locust mixed in the diet (up to 13 weeks; Ochiai et al., 2020) or administered by gavage (28 days; Kwak et al., 2020).

3.10.1. Genotoxicity

The genotoxicity study intended to be performed on *L. migratoria* dried was preceded by solubility and sterility tests.

Samples of *L. migratoria* dried were not soluble in water, DMSO, ethanol and acetone at the lowest concentration of 10 mg/mL, and no homogenous suspension could be achieved. For testing sterility, *L. migratoria* dried was grounded and suspended in sterile water at a concentration of 100 mg/mL. Bacterial colonies and filamentous fungi were observed in two independent tests. The execution of *in vitro* genotoxicity tests was prevented by the lack of solubility of the NF and microbiological contamination.

Genotoxic potential of aqueous extracts (not further specified) of freeze-dried *L. migratoria* was assessed by Turkez et al. (2014) in cultured human lymphocytes by sister chromatid exchange, chromosome aberration and micronucleus assays. The tests were performed without S9 and were negative in concentrations up to 1,000 mg/L. However, the Panel notes that this study bears limitations due to poor reporting regarding the experimental conditions applied and especially the characteristics of the testing material (water extract) with lack of information of insect rearing and processing conditions.

The Panel considers that the available data do not allow to conclude on possible genotoxicity of the NF. Generally, for whole foods, EFSA requests genotoxicity testing with suitable extracts.

The Panel notes that the applicant provided a literature review on the history of human consumption of the insect species. Since no safety concerns were identified in both history of use of *L. migratoria* and compositional data of the NF, the Panel considers that genotoxicity studies are not needed in this specific case.

3.10.2. Cytotoxicity

A cell toxicity assay was performed testing LM powder on three human cell types (HL60 cells, HeLa cells and Caco-2 cells). Cell survival was quantified by a colorimetric test to measure mitochondrial activity in viable cells.

No cytotoxic effect was observed in any concentration of the NF used in the studies up to 250 μg/mL.

3.10.3. Subacute and subchronic toxicity

EFSA identified two papers that provide information on the safety of locust powder after repeated administrations.

In the study by Kwak et al. (2020), 28 days repeated-dose oral toxicity of freeze-dried *L. migratoria* powder was investigated in Sprague-Dawley (SD) rats. Information on insect feed used, rearing conditions and the terminal procedures (fasting, washing, steam sterilisation and freezing) of *L. migratoria* are also described in the paper. No specific composition of the test item was provided in the study. Authors stated that the study has been conducted in agreement with GLP and according to the relevant OECD (Organization for Economic Co-operation and Development) guideline (OECD TG407 of 2008). Freeze-dried powder of *L. migratoria* was administered once a day in distilled water (20 mL/kg) by gavage at the doses of 750, 1,500 and 3,000 mg/kg to five male and five female rats per group. Changes in a few haematological or biochemical parameters (i.e. monocytes and basophils, inorganic phosphorus) have been noted; however, they were of limited magnitude, limited to one sex and without dose correlation. Statistically significant lower prostate weight (relative and absolute) in the high dose has been recorded, however, reported to be within the historical biological range and therefore considered of doubtful biological relevance. The Panel notes that histopathology examination was limited to liver and kidney (no abnormalities were noted) at all doses tested, while other collected organs/tissues were not examined even in the high dose group.

The authors concluded that under the experimental conditions applied, the NOAEL (no observed adverse effect level) was considered the high dose of 3,000 mg/kg bw per day. On the basis of the information available from the paper and with the limitations noted, the Panel agrees on this conclusion.

In Ochiai et al. (2020), acute, subacute and subchronic oral toxicity studies were conducted with powdered *L. migratoria*[5] produced in Thailand. No information was available concerning the insect rearing conditions and processing to obtain the powder. The *L. migratoria* powder utilised in this study contained 75.5% protein, 12.0% fat, 3% ash and 0.5% fibre, compared to the NF specifications (48.5–63.5% protein, 28–42% fat, 1.3–2.8% ash, 5.5–9.0% fibre). The study was not described as conducted according to Good Laboratory Practices (GLP), however, it is stated that experimental procedures applied were following principles of OECD test guidelines (specifically OECD TG 407 and

[5] Confirmation of the species received by EFSA from the study author.

425 of 2008). EFSA noted however that there were several deviations from the OECD guidelines (e.g. too few animals, only males investigated, no histopathological examination).

In subacute and subchronic toxicological test, six male Wistar rats per group were fed with a diet containing 0%, 1% and 3% locust powder for 28 and 90 days. Rats were monitored for general signs of toxicity, body weight, organ weights, haematology and clinical chemistry parameters, lipids in liver and faeces and short chain fatty acids in cecum content. No histopathologic examinations were performed. The subacute toxicological experiment showed a decrease in red blood cells and related parameters at both concentrations of locust powder at the end of the 28 days, but not observed in the subchronic setting (i.e. the 90 days). The authors considered these differences to be a transient event as the plasma levels of iron and other parameters were not significantly changed in both experiments. Glucose in plasma was decreased at 3% locust powder only after 28 days, while insulin in plasma was reduced at 3% locust powder diet at the end of 90 days. Liver lipid accumulation and faecal fat excretion increased in the 3% locust powder diet, but were not considered as an adverse effect in absence of any other toxicologically relevant finding. Gut and cecum content were dose dependently decreased after 28 and 90 days but was statistically significant only for the gut content relative to body weight after 28 days ingestion of diet containing 3% locust powder. As highlighted by the authors, this effect was likely due to enhancement of gastric emptying and intestinal transit. Finally, cecum short-chain fatty acids were decreased by the locust powder, possibly caused by its increased fibre and fibre-like components.

The authors concluded that from the subchronic administration the locust powder when mixed up to 3% in the diet of male rats did not elicit any toxicologically relevant finding. However, considering the deviations from OECD TG with limited number of animals involved, the use of one sex and the absence of relevant investigations (e.g. histopathology) no firm conclusions can be drawn by the NDA Panel.

Although the test items used in the studies described in these papers cannot be considered fully representative of the NF and in spite of some limitations in the experimental designs and in the reporting, the Panel considers that these toxicological studies conducted with *L. migratoria* can be used as supporting evidence for the safety of the NF and do not raise safety concerns.

3.10.4. Human data

No human studies were provided by the applicant, or retrieved from literature, with the NF or its source.

3.11. Allergenicity

A literature search has been carried out by the Applicant using Google Scholar and Scopus® to retrieve relevant data. The relevant information on the source, production process, protein characterisation, reported case studies for allergenicity due to exposure or consumption of *L. migratoria*, immunological studies, cross-reactivity and effect of processing/digestion on allergens have been reported.

Locusta migratoria (family Acrididae) belongs to the subphylum Hexapoda (class Insecta), one of the four subphyla of Arthropoda, the others being Crustacea, Myriapoda and Chelicerata. Within arthropods, several allergens have been reported, including tropomyosin (Reese et al., 1999), arginine kinase (Binder et al., 2001) and glutathione S-transferase (Galindo et al., 2001). Furthermore, chitinases, the enzymes that degrade chitin, have been identified as an allergen in some insect species (Zhao et al., 2015).

Few prevalence studies on food allergy related to insects, mainly for Asian populations, are available (China and Laos) (Ji et al., 2009; Barennes et al., 2015). Sokol et al. (2017) registered two events of anaphylaxis due to ingestion of chapulines (roasted grasshopper from Oaxaca, Mexico) in patients allergic to crustaceans who had no previous exposure to grasshoppers. According to Ji et al. (2009), ingestion of insects was the cause of 18% anaphylactic food-related reactions in China from 1980 to 2007, and locusts accounted for 27 cases out of 358. Furthermore, the occurrence of anaphylactic reactions due to fried insects (grasshoppers and crickets) has also been registered in Thailand (Piromrat et al., 2008).

Multiple allergens have been found in *L. migratoria* extracts of different molecular weight (Tee et al., 1988; Lopata et al., 2005; Ji et al., 2009) using different approaches. By using a proteomic and bioinformatic approach, Barre et al. (2021) identified 73 proteins in *L. migratoria*, corresponding to pan-allergens which develop cross-reactivity with other homologous proteins present in arthropods

(house dust mites and crustaceans), followed by allergens from molluscs and nematodes. These include arginine kinase, chitinase, glutathione S-transferase, HSP 70, hexamerin, serine protease, tropomyosin and trypsin. Tropomyosin appeared as a major pan-allergen largely distributed among dust mites, insects, crustaceans and molluscs, as also confirmed by Sokol et al. (2017).

Cross-reactivity to *L. migratoria* protein extracts has been evidenced by immunoblotting in sera from crustaceans and house dust mite allergic individuals (Pali-Schöll et al., 2019a) and in sera from shrimp allergic individuals (Broekman et al., 2017). The main reason for cross-reactivity is the high protein homology between phylogenetically related organisms, being evident not only between species within the same subphylum, but also between species from different arthropod subphyla. It includes crustacean species (e.g. shrimp, crab), chelicerates (e.g. mites) and several insect species (Santos et al., 1999; Binder et al., 2001; Galindo et al., 2001; Liu et al., 2009; Lopata et al., 2010; Verhoeckx et al., 2014; Van Broekhoven et al., 2016; Rougé and Barre, 2017; Broekman et al., 2017; De Gier and Verhoeckx, 2018).

In addition, food processing per se may also have an influence on allergenicity, and this applies to insect allergens as well, although the effect of food processing on allergenicity cannot be predicted (EFSA NDA Panel, 2014). Pali-Schöll et al. (2019b) reported that some processing treatments, such as enzymatic hydrolysis or thermal treatments reduced the IgE binding from crustacean and mite allergic patients to *L. migratoria* (immunoblotting and skin prick test). de Gier and Verhoeckx (2018) reported a review of the literature on the effect of thermal processing on allergenicity of several insect proteins and concluded that it did not eliminate insect protein allergenicity.

Additional aspects should be taken into consideration depending on the feed substrate used to rear *L. migratoria*, as it might include common allergenic foods. The applicant reported that a plant-based substrate containing gluten was used as feed, hence traces of gluten may be found in the insects' gut. The Panel notes that changes in the feed can possibly introduce additional allergens, including allergens which require mandatory labelling according to Annex II of Regulation (EU) No 1169/2011, since traces of the allergens may remain in the gut of *L. migratoria* despite implementing a fasting step.

A frequently reported case of allergic symptoms to insects, including *L. migratoria*, relates to occupational exposure (Burge et al., 1980; Tee et al., 1988; Lopata et al., 2005).

The Panel considers that the consumption of the NF might trigger primary sensitisation to *L. migratoria* proteins. The Panel also considers that allergic reactions may occur in subjects allergic to crustaceans, mites and molluscs (cross-reactivity). Furthermore, Panel notes that additional allergens may end up in the NF, if these allergens are present in the substrate fed to the insects.

4. Discussion

The NF which is the subject of the application is migratory locust (*Locusta migratoria*), frozen without legs and wings, dried without legs and wings, or in the form of powder (with legs and wings). The production process is sufficiently described and does not raise safety concerns. The Panel considers that the NF is sufficiently characterised. The NF consists mainly of protein, fat, dietary fibre (mainly chitin) and inorganic matter (LM dried and LM powder); water, protein, fat, dietary fibre (mainly chitin) and inorganic matter (LM frozen). The concentrations of contaminants in the NF depend on the occurrence of these substances in the insect feed. Provided that applicable EU legislation regarding feed is followed, the consumption of the NF does not raise safety concerns. The Panel notes that there are no safety concerns regarding stability if the NF complies with the proposed specification limits during its entire shelf life.

The applicant intends to market the NF as an ingredient in several food products. The target population is the general population. Intake was estimated based on the use of the NF as an ingredient in the intended food categories at the maximum proposed levels across surveys in the EFSA Comprehensive European Food Consumption Database. The highest intake estimate was calculated for young children (1–< 3 years old) ranging from 176 to 1370 mg NF/kg bw per day at the 95th percentile of the intake distribution. The Panel notes that consumption of the NF under the proposed uses and use levels does not contribute substantially to the total dietary exposure to the analysed undesirable substances.

The Panel notes that the dried formulations of the NF have a high protein content, although the true protein levels in the NF are overestimated due to the presence of non-protein nitrogen of chitin when using the conversion factor of 6.25. The true ileal nitrogen digestibility of the NF LM powder (LM dried with legs and wings) is 55.4% ± 2.4%, with a DIAAS value of 70%. The limiting amino acids were the sulfur-containing ones. None of the existing upper levels for the analysed micronutrients are

exceeded considering the proposed uses and use levels. The reported concentrations of the antinutritional factors in the NF are comparable to those in other foodstuffs. The Panel considers that the main type of fibre in the NF, chitin, is an insoluble fibre not expected to be digested in the small intestine of humans to any significant degree and is assumed to be excreted mainly unchanged. Additionally, the Panel notes that chitin, like other fibres, can possibly affect the bioavailability of minerals. The Panel notes that, taking into account the composition of the NF and the proposed conditions of use, consumption of the NF is not nutritionally disadvantageous. Considering that no adverse effects were observed in the toxicological studies available in the literature on *L. migratoria*, the history of use of the NF and its source, the Panel considers that there are no safety concerns.

The Panel considers that the consumption of the NF might trigger primary sensitisation to *L. migratoria* proteins. The Panel also considers that allergic reactions may occur in subjects allergic to crustaceans, mites and molluscs (cross-reactivity). Additionally, the Panel notes that allergens from the feed (e.g. gluten) may be present in the NF.

5. Conclusions

The Panel concludes that the NF is safe under the proposed uses and use levels. In addition, the Panel notes that allergic reactions are likely to occur.

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 (description of the production process, analytical data on the composition of the NF, analytical data on contaminants in the NF, stability and microbiological status, data on NF sales, intake assessment, protein digestibility and DIAAS, genotoxicity and cytotoxicity study).

6. Recommendation

The Panel recommends that research should be undertaken on the allergenicity to *Locusta migratoria*, including cross-reactivity to other allergens.

7. Steps taken by EFSA

1) On 09/08/2019 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of whole and ground grasshoppers (*Locusta migratoria*) as a novel food Ref. Ares(2019)5172695.
2) On 09/08/2019, a valid application on whole and ground grasshoppers (Locusta migratoria), which was submitted by Fair Insects BV (A Protix Company), was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2018/0803) and the scientific evaluation procedure was initiated.
3) On 31/08/2020, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 21/09/2020, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) On 16/11/2021, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
6) On 16/01/2021, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
7) On 12/02/2021, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
8) On 13/04/2021, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
9) On 20/04/2021, EFSA received a letter from the European Commission with the revised request for a scientific opinion on frozen and dried formulations from migratory locust (*Locusta migratoria*) as a novel food.

10) During its meeting on 25/05/2021, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of frozen and dried formulations from migratory locust (*Locusta migratoria*) as a NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
AMCase	acidic mammalian chitinase
ANF	antinutritional factors
ASL	accelerated conditions
BIOHAZ	EFSA Panel on Biological Hazards
bw	body weight
CFU	Colony Forming Units
CONTAM	EFSA Panel on Contaminants in the Food Chain
CrIV	cricket iridovirus
DIAAS	digestible indispensable amino acid score
DMSO	dimethyl sulfoxide
FAO	Food and Agriculture Organization
FFA	free fatty acids
GC-MS/MS	gas chromatography coupled mass spectrometry
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practice

HACCP	hazard analysis critical control points
IAC-LC/FLD	immunoaffinity chromatography-liquid chromatography/fluorescence detector
ICP-MS	inductively coupled plasma-mass spectrometry
ICP-OES	inductively coupled plasma atomic emission spectroscopy
IgE	immunoglobulin E
LC-MS/MS	liquid chromatography/tandem mass spectrometry
LM	*Locusta migratoria*
LOAEL	lowest observed adverse effect level
LOQ	limits of quantification
MRL	maximum residue limits
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NF	novel food
NOAEL	no observed adverse effect level
NVWA	Dutch Food and Consumer Product Safety Authority
OECD	Organization for Economic Co-operation and Development
PA	P-anisidine value
PAH	polycyclic aromatic hydrocarbons
PV	peroxide value
SD	Sprague Dawley
WHO (2005) PCDD/F+PCB TEQ	sum of polychlorinated dibenzo-para-dioxins-polychlorinated dibenzofurans-polychlorinated biphenyls expressed as World Health Organization toxic equivalent

Appendix A – Amino acid profile (mg/g protein) of LM powder*

Amino acids (mg/g true protein)	LMDNFD01	LMDNFD02	LMDNFD03	LMDNFD04	LMDNFD05	Average	FAO (2013b)
Essential							
Histidine[1]	26.3	26.2	25.9	26.8	26.5	26.4	15
Isoleucine[1]	44.4	44.7	44.7	44.5	43.9	44.4	30
Leucine[1]	85.6	85.8	85.5	85.6	85.6	85.6	59
Lysine[1]	55.7	56.4	57.9	56.9	54.7	56.3	45
Methionine[1]	14.9	14.9	15.3	15.1	14.5	14.9	22[3]
Phenylalanine[1]	33.0	33.0	33.7	33.0	31.8	32.9	38[4]
Threonine[1]	39.9	41.3	42.3	41.3	40.7	41.1	23
Tryptophan[2]	10.6	10.4	9.8	10.5	10.2	10.3	6
Valine[1]	70.4	69.8	69.7	70.2	71.4	70.3	39
Conditionally essential							
Arginine[1]	59.1	59.4	60.2	59.0	58.8	59.3	
Cysteine + cystine[1]	8.3	8.2	7.7	8.2	8.0	8.1	
Glycine[1]	69.8	69.5	68.4	69.8	69.8	69.5	
Proline[1]	73.1	73.8	69.1	71.4	75.5	72.6	
Tyrosine[1]	53.1	53.7	52.7	53.0	54.0	53.3	
Alanine[1]	127.7	127.6	125.4	128.0	134.1	128.6	
Aspartic acid[1]	84.5	82.9	85.7	84.4	81.7	83.8	
Glutamic acid[1]	103.2	100.9	104.3	101.4	98.2	101.6	
Serine[1]	40.3	41.5	41.7	40.8	40.5	41.0	

*: Analyses performed on LM dried including legs and wings.
1: Method ISO 13903:2005.
2: Method EU 152/2009.
3: Methionine + cysteine.
4: Phenilalanine + tyrosine.

Appendix B – Fatty acid profile of major fatty acids (% fatty acids) in LM powder*

Fatty acids	LMDNFD01	LMDNFD02	LMDNFD03	LMDNFD04	LMDNFD05
Palmitic acid	28.1	28.5	28.0	28.8	29.2
Stearic acid	7.1	7.1	7.2	7.3	7.2
Oleic acid	36.9	37.4	36.8	39.5	38.5
Linoleic acid	11.8	11.3	11.9	8.9	9.8
Alpha- linolenic acid	11.2	11.0	11.2	10.7	10.8
Saturated fatty acid	37.9	38.0	37.8	38.8	38.7
MUFA	39.0	39.5	38.9	41.5	40.5
PUFA	23.1	22.4	23.3	19.7	20.8
Omega 3	11.3	11.1	11.3	10.8	10.9
Omega 6	11.8	11.3	12.0	8.9	9.8
Trans Fatty acids	0.4	0.4	0.4	0.4	0.4

*: Analyses performed on LM dried including legs and wings.

Annex A – Dietary exposure estimates to the Novel Food for each population group from each EU dietary survey

Information provided in this Annex is shown in an Excel file (downloadable at https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2021.6667#support-information-section).