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## Safety of partially defatted house cricket (*Acheta domesticus*) powder 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 defatted house cricket (*Acheta domesticus*) powder as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is proposed as dry powder. The main components of the NF are protein, fat and fibre (chitin). 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 further 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 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 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 Panel notes that no genotoxicity and no subchronic toxicity studies with the NF were provided by the applicant. Considering that no safety concerns arise from the history of use of the source of the NF (*A. domesticus*), and the limited difference between the whole insect and the partially defatted NF, the Panel identified no other safety concerns than allergenicity. The Panel considers that the consumption of the NF might trigger primary sensitisation to *A. domesticus* proteins and may cause allergic reactions in subjects allergic to crustaceans, mites and molluscs. Additionally, allergens from the feed may end up in the NF. That aside, the Panel concludes that the NF is safe under the proposed uses and use levels.

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**Keywords:** Novel Foods, food safety, *Acheta domesticus*, house cricket, insect powder

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## 1. Introduction

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

On 24 July 2019, the company Cricket One Co., Ltd, submitted a request to the Commission in accordance with Article 10 of Regulation (EU) No 2015/2283 to place on the EU market defatted whole cricket (*Acheta domesticus*) powder.

The application requests to authorise the use of defatted whole cricket (*Acheta domesticus*) powder in a number of foods. The target population is the general population above the age of three years. The applicant has requested data protection according to provisions of 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 defatted whole cricket (*Acheta domesticus*) powder.

In addition, the European Food Safety Authority is requested to include in its scientific opinion a statement as to if, if so to what extent, the proprietary data for which the applicant is requesting data protection was used in elaborating the opinion in line with the requirements of Article 26(2)(c) of Regulation (EU) 2015/2283.

In the process of the evaluation of this Novel Food, it became apparent that the Commission should amend the title of the mandate. The term "whole" does not reflect the nature of the Novel Food as this is obtained after fractionation of lipids. Furthermore, the term "cricket" is generic and does not exclusively refer to *A. domesticus*. On that basis, the Commission amended the title to "Revised request for a scientific opinion on defatted house cricket (*Acheta domesticus*) powder as a novel food".

### 1.2. Interpretation of the Terms of Reference

Given the proposed intended uses and in accordance with 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 NDA Panel notes the content of fat in the NF (9–12% as set in the specifications) and considers that the use of the wording 'partially defatted' instead of 'defatted' is more appropriate to describe the NF.

### 1.3. Additional information

On 7 July 2021, the NDA Panel adopted an opinion on the safety of frozen and dried formulations from whole house crickets (*A. domesticus*) as a novel food pursuant to Article 10 of Regulation (EU) 2015/2283. The Panel concluded that the NF is safe for human consumption under the proposed uses and use levels. Following a positive vote of the Standing Committee on Plants, Animals, Food and Feed, the European Commission adopted on 10 February 2022 the Commission Implementing Regulation (EU) 2022/188 authorising the placing on the market of frozen, dried and powder forms of *A. domesticus* as NF pursuant to Regulation (EU) 2015/2283.

## 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 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 UCT Prague (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<sup>1</sup>.

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<sup>1</sup> 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.

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: description of the production process, analytical data on the composition of the NF, analytical data of amino acid and fatty acids, protein digestibility.

## 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 partially defatted powder of *A. domesticus* (house cricket). The NF falls under the category of 'food consisting of, isolated from or produced from animals or their parts', as described in Article 3 (v) of Regulation (EU) 2015/2283. The NF is produced by farming and processing of *A. domesticus* and consists mainly of protein, fat and fibre (dry basis). The NF is proposed to be marketed in the form of powder. The applicant proposed to use the NF as ingredient in various food products. Products with the NF can be consumed by the general population.

### 3.2. Identity of the NF

The NF consists of partially defatted dried powder of house cricket. The term 'house cricket' refers to *A. domesticus*, an insect species that belongs to the family of Gryllidae, subfamily Gryllinae, genus *Acheta*. The species is present in various regions worldwide, including Australia, Asia, Africa, North America and Europe (GBIF Secretariat, 2021). The identity of the species has been certified by a National official inspection performed to issue the business licence under the Husbandry Law of Vietnam, Decree 13/2020/ND-CP, Appendix VIII. The insects are farmed under controlled rearing conditions.

### 3.3. Production process

According to the information provided, the NF is produced in line with Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Points (HACCP) principles. The applicant stated that insects were reared at a facility qualified with Husbandry Conditions for Food by the Department of Agriculture and Rural Development of Lộc Ninh City, Bình Phước province (Vietnam). The production process can be divided into three distinctive parts, i.e. farming, harvesting and post-harvest processing.

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 regularly disinfected containers made of certified food-contact polypropylene. Insects are reared in multiple production plants owned by the applicant and operating under the same conditions. The applicant reported that no pesticides, antibiotics or solvents are used during the entire production process, and that no growth hormones are used during farming.

The applicant reported that the feed used to feed *A. domesticus* is a plant-derived material compliant with Directive 2002/32/EC. The applicant reported the presence of GMO corn flour in the feed mixture and commits to inform the consumers about the presence of GMO ingredient in the feed, according to Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22

September 2003 concerning the traceability and labelling of genetically modified organisms, and the traceability of food and feed products produced from genetically modified organisms.

The applicant indicated that cassava parts (leaves, stem and branches) may be included in the feed. The presence of hydrocyanic acid has been discussed under Section 3.7.4 – estimate of exposure to undesirable substances.

During farming, *A. domesticus* can be affected by pathogens including cricket paralyzes virus (CrPV) of the Dicistroviridae family, the cricket densovirus (AdDV) from the Parvoviridae family (Maciel-Vergara and Ros, 2017), *Penaeus merguensis* densovirus (Pmerg DNV) (La Fauce and Owens, 2008) and the nematode *Heterorhabditis georgiana* (Shapiro-Ilan et al., 2009). Literature review conducted by the applicant highlighted that these pathogens are specific for insects, and non-pathogenic for humans or other vertebrates. Examples of food-borne bacteria that may be present in *A. domesticus* include *Citrobacter*, *Klebsiella* and *Yersinia* (Fernandez-Cassi et al., 2020). However, their potential presence in the NF is monitored by microbiological analysis of Enterobacteriaceae as reported in Section 3.4, Table 4. Decayed insects are removed after visual inspection throughout rearing.

Adult insects are harvested (5–7 weeks old) after being separated from the substrate and faeces. Adults are transported alive at a temperature < 25°C to the processing facility (within 20 km distance). Then, a 24-h fasting step is implemented to allow the insects to discard their bowel content, followed by freezing at –18°C to kill the insects.

The post-harvesting process includes multiple washing steps in running water and salted water (0.5% sodium chloride) to remove foreign matters, blanching in hot water (100°C for at least 3 min), dehydration in hot air circulating chamber (< 90°C for at least 6 h), oil extraction (mechanical extrusion < 65°C) and grinding. Sieving is finally performed to obtain the NF 'partially defatted cricket powder' of particle size < 0.17 mm. The thermal treatment contributes to the reduction of the microbiological load of the insects as well as to the elimination of potentially present viruses and parasites, and reduction of enzymatic activity. Dehydration of the insects takes place in hot air circulating chambers, resulting in a final product with moisture content < 6 g/100 g. The NF is stored in vacuum-heat sealed packaging at room temperature.

The Panel considers that the production process is sufficiently described.

### 3.4. Compositional data

The applicant provided qualitative and quantitative data on chemical and microbiological parameters for different batches of the NF. For all parameters, five independently produced batches were analysed. Certificates of accreditation for the laboratories that conducted the analyses were provided by the applicant. Analytical data were produced using methods validated for other types of matrices and a full description of the methods has been provided.

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

**Table 1:** Proximate analysis of the NF

Parameter (unit)	Batch number					Analytical method
	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	
Crude protein (g/100 g)	74.4	74.1	74.2	77.2	76.8	TVCN 4328-1:2007 (ISO 5983-1:2005) Kjeldahl, (N × 6.25)
Fat (g/100 g)	9.4	9.2	9.2	11.6	11.5	TCVN 4331: 2001 (ISO 6492:1999) Hydrolysis/gravimetry
Crude fibre (g/100 g)	9.1	9.2	9.6	8.2	8.1	TCVN 4329: 2007; (ISO 6865:2000) gravimetry
Total carbohydrate (g/100 g)	5.0	5.3	5.8	3.2	3.8	Calculation*
Ash (g/100 g)	5.5	5.6	5.0	4.6	4.3	AOAC 942.05
Moisture (g/100 g)	5.7	5.7	5.8	3.4	3.5	AOAC 925.10:2008

Parameter (unit)	Batch number					Analytical method
	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	
Sugars (g/100 g)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	TCVN 4594:1988 Fehling reagent
Energy value (kcal/100 g)	350	350	350	380	380	TCVN 8762:2012 Regulation (EU) 1169/2011

NF: novel food; LOQ: limit of quantification; LOQ sugars: 0.1 g/100 g; TCVN: Vietnam Standards; \* 100 – moisture, protein, ash and fat; AOAC: Association of official analytical collaboration; ISO: International Organization for Standardization.

The Panel notes that there is a variation of the values of some proximate parameters, but this can be expected since the NF is produced using whole insects. The values may also depend on the rearing conditions (feed, developmental stage at the time of harvesting, ambient conditions) (Rumpold and Schlüter, 2013; Oonincx et al., 2015).

Regarding the crude protein content of the NF, the Panel notes that recent literature (Boulos et al., 2020) suggests that it is likely 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 Nutritional information'.

Chitin is a linear polysaccharide constituted by  $\beta$ -(1,4)-linked 2-amino-2-deoxy- $\beta$ -D-glucopyranose and 2-acetamido-2-deoxy- $\beta$ -D-glucopyranose residues (Roberts, 1992). The physicochemical nature of chitin is intrinsically related to its source (Kumirska et al., 2011). The applicant provided analytical data on the levels of chitin in five independently produced batches of the NF (Table 2). The Panel notes that a nationally or internationally recognized reference method for the analytical determination of chitin does not exist. The chitin content in the NF was determined 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 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 NF

Chitin (g/100 g NF)	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5
ADF (g/100 g) <sup>(a)</sup>	7.3	6.8	7.0	8.7	8.7
ADL (Lignin) (g/100 g) <sup>(b)</sup>	2.4	1.7	1.8	0.6	0.7
Chitin (g/100 g) <sup>(c)</sup>	4.9	5.1	5.2	8.1	8.0

NF: novel food; ADF: acid detergent fibre (AOAC 973.18).

Lignin: AOAC 973.18 Lignin (H<sub>2</sub>S O<sub>4</sub>); ADL: acid detergent lignin.

Chitin calculated as ADF-Lignin.

Analytical data on the concentrations of heavy metals, dioxins and dioxin-like polychlorinated biphenyls (PCBs), aflatoxins B1, B2, G1, G2, ochratoxin A, nivalenol, deoxynivalenol, in the NF were provided by the applicant. Analytical data on fumonisin B1, fumonisin B2 and zearalenone were provided upon EFSA's request (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 concentrations of contaminants reported for the NF are lower than maximum levels set for other foods, and that in the current EU legislation, no maximum levels of these contaminants are set for insects as food.

**Table 3:** Heavy metal, mycotoxin and dioxins levels in the NF

Parameter	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	Analytical method
<b>Heavy metals (mg/kg)</b>						
Arsenic (As)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	AOAC 986.15:2005 modified AAS
Mercury (Hg)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	AOAC 974.14:2005 modified AAS
Lead (Pb)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	AOAC 999.10:2005 modified AAS
Cadmium (Cd)	< 0.025	< 0.025	< 0.025	< 0.025	< 0.025	

Parameter	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	Analytical method
<b>Mycotoxins (<math>\mu\text{g}/\text{kg}</math>)</b>						
Aflatoxins B1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	DIN EN 14123:2008-03 HPLC-post-column derivatization/IAC
Aflatoxins B2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
Aflatoxins G1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
Aflatoxins G2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
Aflatoxins (Sum of B1, B2, G1, G2)	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	
Ochratoxin A	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	AOAC 2001 84.6.10 RPLC/FD
Deoxynivalenol	< 10	< 10	< 10	< 10	< 10	FST-WI01 chapter 39 LC-MS/MS
Zearalenone	< 10	< 10	< 10	< 10	< 10	FST-WI01 chapter 72 LC-MS/MS
Fumonisin B1	< 100	< 100	< 100	< 100	< 100	
Fumonisin B2	< 100	< 100	< 100	< 100	< 100	
<b>Dioxins (pg/g fat)</b>						
WHO (2005) PCDD/F-TEQ (upper bound)	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	EPA 1613 HRGC-HRMS

AAS: atomic absorption spectrometry; LC-MS/MS: liquid chromatography-tandem mass spectrometry; HRGC: high-resolution gas chromatography; HRMS: high-resolution mass spectrometry; HPLC high-performance liquid chromatography; IAC: immunoaffinity column cleanup; DIN: Deutsches Institut für Normung; EN: Europäische Norm; EPA: Environmental Protection Agency; RPLC/FD: reversed-phase liquid chromatography/fluorescence detector; AOAC: Association of official analytical collaboration; FST-WI: Phương pháp do phòng thí nghiệm xây dựng/Laboratory developed method; WHO (2005) PCDD/F-TEQ: sum of polychlorinated dibenzo-p-dioxins-polychlorinated dibenzofurans-polychlorinated biphenyls expressed as World Health Organization toxic equivalent.

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 NF are below the limits of quantification (LOQs) of the implemented methods (FST-WI06 chapter 03, Ref. AOAC 985.22:2005 & AOAC 2007.01.2013, GC/ $\mu\text{ECD}$ ), (FST-WI06 chapter 10, GC/ $\mu\text{ECD}$ ) (FST-WI06 chapter 21, Ref. AOAC 2007.01 2013, GC/MS).

Given the vegetable origin of the substrate and the absence of prion or prion-related encoding genes in insects, 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 biogenic amines for five independently produced batches of the NF at  $t = 0$  of the shelf life. Average histamine level was 34.6 mg/kg (AOAC 977.13 – LC-MS/MS). High concentration levels of spermidine (142 mg/kg) and putrescine (57.6 mg/kg) were reported. No legal maximum levels have been established for spermidine and putrescine in foods. A similar concentration of spermidine has been reported in *A. domesticus* (150 mg/kg) (EFSA NDA Panel, 2021), and spermidine and putrescine have been detected respectively in legumes (206 mg/kg and 46.3 mg/kg) and cereals (353 mg/kg and 62.0 mg/kg) (Muñoz-Esparza et al., 2019). Formation of biogenic amines can occur by endogenous biosynthesis, uptake from the feed source and by bacteria of the intestinal microflora 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 analysed the NF for *Pseudomonas aeruginosa* which belongs to *Pseudomonas* genus and could have contributed to the occurrence of biogenic amines in the NF. *P. aeruginosa* was reported at levels < 10 cfu/g in five independently produced batches of the NF.

The applicant provided microbiological data on five independently produced batches of the NF. The Panel notes that the microbiological values of the analysed samples do not exceed the specification limits.

**Table 4:** Batch-to-batch microbiological analyses of the NF

Parameter	Unit	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	Analytical method
Total aerobic count	cfu/g	40,000	53,000	51,000	14,000	10,000	ISO 4833-1:2013
Enterobacteriaceae	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 21528-2:2017
<i>Escherichia coli</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 16649-2:2001



Parameter	Unit	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	Analytical method
<i>Listeria monocytogenes</i>	in 25g	ND	ND	ND	ND	ND	ISO 11290-1:2017
<i>Salmonella</i> spp.	in 25g	ND	ND	ND	ND	ND	ISO 6579-1:2017
<i>Bacillus cereus</i>	cfu/g	70	80	80	70	80	ISO 7932:2004
Coagulase-positive staphylococci	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 6888-1:1999/Amd1:2003
<i>Clostridium perfringens</i> - vegetative form	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 7937:2004
<i>Clostridium perfringens</i> - spore form	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 7937:2004
Yeast	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 21527/2:2008
Moulds	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 21527/2:2008

cfu: colony forming units; ND: not detected; ISO: International Organization for Standardization.

The Panel considers that the 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 NF (Table 5). The NF has been analysed after 12 months at room temperature in vacuum-heat-sealed packaging.

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

Parameter	Unit	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	Analytical method
Total aerobic count	cfu/g	5,000	7,200	7,000	910	1,300	ISO 4833-1:2013
Enterobacteriaceae	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 21528-2:2017
<i>Escherichia coli</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 16649-2:2001
<i>Listeria monocytogenes</i>	in 25g	ND	ND	ND	ND	ND	ISO 11290-1:2017
<i>Salmonella</i> spp.	in 25g	ND	ND	ND	ND	ND	ISO 6579-1:2017
<i>Bacillus cereus</i>	cfu/g	40	40	10	200	220	ISO 7932:2004
Yeast	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 21527/2:2008
Moulds	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 21527/2:2008

cfu: colony forming units; ND: not detected; ISO: International Organization for Standardization.

The applicant indicated that the high content of *B. cereus* in Colony 4 and Colony 5 (Table 5) may be due to loss of vacuum during storage, and that more strict quality control plan will be applied to ensure values are below specifications.

After EFSA's request, the applicant provided analytical data on the oxidative status of the lipids and proteins degradation during the shelf life (Table 6). The data provided (temperature < 27°C, RH < 70%) cover a period of at least 12 months which is the proposed shelf life.

**Table 6:** Oxidative status of fat and protein degradation in the NF during the proposed shelf life

Time (months)	0					12					Analytical methods
	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	
Free fatty acids (% wt)	1.8	1.9	1.7	1.9	1.7	1.1	1.1	1.1	1.8	1.7	ISO 660:2009
Peroxide value (meq O <sub>2</sub> /kg fat)	1.1	0.9	0.8	ND	ND	0.6	0.4	0.4	ND	ND	ISO 3960:2017
<i>p</i> -Anisidine value	–	–	–	–	–	1.7	1.7	2.6	1.0	2.7	ISO 6885:2000

Time (months)	0					12					Analytical methods
	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	
Total volatile basic nitrogen (mg/100 g)	70	73	78	56	73	41	40	41	77	73	Commission Regulation (EC) No 2074 (2005)

meq: milliequivalents; ND: not detected; ISO: International Organization for Standardization.

### 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 the stability when used as an ingredient in the intended-for-use matrices (see Section 3.7.2 Proposed uses and use levels). The applicant examined the lipid hydrolysis and oxidation, the formation of processing contaminants and microbiological content of 'biscuits' and microbiological content of 'sausage'. Data were provided for five independently produced batches of each product analysed after production (Table 7).

**Table 7:** Stability of the NF in intended for use matrices

Parameter	Unit	Control	Batch1	Batch2	Batch3	Batch4	Batch5	Analytical method
<b>Biscuits</b>								
Free fatty acid	g/100 g	0.17	0.25	0.28	0.32	0.31	0.29	Ref. TCVN 6127:2010 (ISO 660:2009) titrimetric
Peroxide value <sup>a</sup>	meqO <sub>2</sub> /kg	ND	ND	ND	ND	ND	ND	FST-WI08 Chapter 49 (Ref.TCVN 6121:2018) (ISO 3960:2017) iodometric
Acrylamide <sup>b</sup>	µg/kg	ND	ND	ND	ND	ND	ND	FST-UI01 Chapter 79 (LC-MS/MS)
3MCPD <sup>c</sup>	mg/kg	ND	ND	ND	ND	ND	ND	CASE.SK.0015 (GC-MS/MS)
<b>Microbiological quality</b>								
Total aerobic count (30°C)	cfu/g	< 10	250	50	50	90	60	ISO 4833-2:2013/ Cor1:2014
<i>Escherichia coli</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	ISO 16649-2: 2001
<i>Listeria monocytogenes</i>	in 25 g	ND	ND	ND	ND	ND	ND	ISO 11290-1:2017
<i>Salmonella</i> spp.	in 25 g	ND	ND	ND	ND	ND	ND	ISO 6579-1:2017/ Amd1:2020
<i>Bacillus cereus</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	ISO 7932: 2004/ Amd1:2020
<b>Sausage</b>								
Microbiological quality								
Total aerobic count (30°C)	cfu/g	3,300	2,500	2,100	2,100	2,200	2,700	ISO 4833-2:2013/ Cor1:2014
<i>Escherichia coli</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	ISO 16649-2: 2001
<i>Listeria monocytogenes</i>	in 25 g	ND	ND	ND	ND	ND	ND	ISO 11290-1:2017
<i>Salmonella</i> spp.	in 25 g	ND	ND	ND	ND	ND	ND	ISO 6579-1:2017/ Amd1:2020

Parameter	Unit	Control	Batch1	Batch2	Batch3	Batch4	Batch5	Analytical method
<i>Bacillus cereus</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	ISO 7932: 2004/ Amd1:2020

LOQ<sup>a</sup>: 0.3 meqO<sub>2</sub>/kg; LOQ<sup>b</sup>: 10 µg/kg; LOQ<sup>c</sup>: 0.01 mg/kg; meq: milliequivalents; cfu: colony forming units; 3MCPD: 3-monochloropropane-1,2-diol or 3-chloropropane-1,2-diol; ISO: International Organization for Standardization; TCVN: Vietnamese standards; FST-WI: Phương pháp do phòng thí nghiệm xây dựng/ Laboratory developed method; CASE.SK: Center for Analysis Service of Experiment of HCMC; GC-MS/MS: gas chromatography coupled tandem mass spectrometry; ND: not detected.

The biscuits were a mixture of wheat flour, butter, sugar, eggs, partially defatted cricked powder (12 g/100 total ingredients), custard powder and salt. After mixing and forming, the biscuits were baked at 185°C for 15 min, cooled, packed in polypropylene bags and stored at room temperature. A control sample was prepared by replacing the NF with wheat flour.

The sausage was a mixture of pork meat, pork lard, water, partially defatted cricket powder (11 g/100 g total ingredients), nitrite salt, converted starch, soy protein, garlic spices, sugar and spices. After mixing and forming, the sausage was thermally processed at 85°C for 45 min, cooled, packed under vacuum in polypropylene (PP) bags and stored at –18°C before analysis. A control sample was prepared by replacing the NF with pork meat and water.

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. The Panel notes that the food items containing the NF have to comply with existing legislative limits, such as microbiological levels established by Regulation (EC) 2073/2005 and the 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 shelf life, and that products containing the NF are compliant with respective legislative limits on process-formed contaminants and microbiological criteria, the stability data do not raise safety concerns.

### 3.5. Specifications

The specifications of the NF are indicated in Table 8.

**Table 8:** Specifications of the NF

<b>Description:</b> thermally processed, partially defatted, dried, ground <i>Acheta domesticus</i> powder		
<b>Source:</b> <i>Acheta domesticus</i>		
Parameter	Unit	Specification
Crude protein	% w/w	74–78
Fat	% w/w	9–12
Saturated fat	% w/w	3.5–5.0
Moisture	% w/w	3–6
Dietary fibre	% w/w	8–10
Chitin*	% w/w	4.0–8.5
Peroxide value	meq O <sub>2</sub> /kg fat	≤ 5
Manganese	mg/kg	≤ 100
Cyanide	mg/kg	≤ 5
<b>Heavy metals</b>		
Lead	mg/kg	≤ 0.1
Cadmium	mg/kg	≤ 0.025
<b>Mycotoxins</b>		
Aflatoxins (Sum of B1, B2, G1, G2)	µg/kg	≤ 0.4
Deoxynivalenol	µg/kg	≤ 10
Ochratoxin A	µg/kg	≤ 0.5
<b>Sum of dioxins and dioxins-like PCBs (UB WHO<sub>2005</sub> PCDD/F-PCB-TEQ)</b>	pg/g fat	≤ 1.25

<b>Microbiological</b>		
TAMC	cfu/g	< 10 <sup>5</sup>
Enterobacteriaceae (presumptive)	cfu/g	≤ 100
<i>Escherichia coli</i>	cfu/g	≤ 50
<i>Listeria monocytogenes</i>	in 25 g	Not detected
<i>Salmonella</i> spp.	in 25 g	Not detected
<i>Bacillus cereus</i> (presumptive)	cfu/g	≤ 100
Coagulase-positive staphylococci	cfu/g	≤ 100
TYMC	cfu/g	≤ 100

TAMC: total aerobic microbial count; TYMC: total yeast and mould count; cfu: colony forming units; UB: upper bound; WHO-PCDD/F-PBC-TEQ: sum of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, polychlorinated biphenyls expressed as World Health Organization toxic equivalent; w/w: weight per weight; ND: not detected.

\*: Chitin calculated as the difference between the Acid Detergent Fibre fraction and the Acid Detergent Lignin fraction (ADF-ADL), as described by Hahn et al. (2018).

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

*A. domesticus* either collected from the wild or reared in farms is consumed as part of the customary diet in some non-EU countries worldwide. Their consumption by humans has been reported mainly in Thailand (Hanboonsong et al., 2013; Yen, 2015) followed by Lao PDR (Codex Alimentarius Commission, 2010; Durst and Hanboonsong, 2015), Cambodia (FAO, 2013), Ghana (Anankware et al., 2016), Mexico (Ramos-Elorduy, 2009) Democratic Republic of Congo and Kenya (Halloran et al., 2018).

Hanboonsong et al. (2013) reported that around 20,000 *A. domesticus* small- and medium-size farms are registered in Thailand. Products are distributed to wholesalers and local markets. Commercial chicken feed and vegetables are used as substrate and 7,500 tonnes of crickets (including *A. domesticus*) a year are produced. In 2017, the Thai Agricultural Standards Committee established Good Agricultural Farming Practices for cricket farming including *A. domesticus* (ACFS, 2017).

*A. domesticus* is also farmed in Lao PDR (Hanboonsong and Durst, 2014), as well as at a lesser extent in Cambodia, Democratic Republic of Congo and Kenya (Halloran et al., 2018).

Additionally, in Australia and New Zealand, it is considered as non-traditional, not novel foodstuff and no safety concerns were identified with the exception of potential risk of allergenicity in crustacean-allergic or other sensitive individuals when consuming crickets or foods derived from crickets (FSANZ, 2021). Since 1 May 2017, *A. domesticus* in adult phase is among the insect species that can be legally introduced in the Swiss market as food (whole, chopped or ground). In Canada, it is considered non-novel for use as a food or food ingredient (Health Canada, 2021).

*A. domesticus* also appears to be marketed for human consumption in the EU, Australia and USA as a whole insect or as a food ingredient in a number of food products (e.g., nutritional bars, lollipops, flour, chocolate. etc.).

The Commission Implementing Regulation (EU) 2022/188 of 10 February 2022 authorised the placing on the market of frozen, dried and powder forms of *A. domesticus*.

#### 3.6.2. History of use of the NF

According to the information provided by the applicant, the NF is already on the market for human consumption in different countries. These include the Netherlands, United Kingdom, United States from 2018, Germany, Australia and Japan from 2019, the Czech Republic, Spain, Denmark, Canada, from 2021, where between 50 and 500 kg of NF per month have been available in the markets. No adverse effects were reported in literature.

### 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 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 9.

**Table 9:** Food categories and maximum use levels intended by the applicant

FoodEx2 level	FoodEx2 code	Food category	Max use level (g NF/100 g)
4	A005K	Bread and rolls with special ingredients added	2
3	A005Y	Crackers and breadsticks	2
3	A00EY	Cereal bars	3
4	A009X	Biscuits, sweet, plain	1.5
5	A007L	Dried pasta	0.25
5	A007Y	Dried stuffed pasta	3
4	A0CSK	Pre-mixes (dry) for backed products	3
5	A03VH	Potatoes and vegetables meal	1
5	A03VN	Hummus	1
5	A03ZT	Pizza and similar with cheese, and vegetable	1
5	A0CDP	Pasta, filled, cooked	1
4	A02PN	Whey powder	3
3	A03TE	Meat imitates	5
4	A0B9R	Mixed vegetable soup, dry	1
4	A042E	Caesar salad	1
4	A042H	Prepared pasta salad	1
5	A00FD	Tortilla chips	4
2	A03MA	Beer and beer-like beverage	0.1
4	A0EQD	Chocolate and similar	2
3	A01BJ	Primary derivatives from nuts and similar seeds	2
3	A06HL	Snacks other than chips and similar	5
5	A03XG	Meat balls	2
5	A03XF	Meat burger (no sandwich)	2
4	A024J	Fresh spiced sausage	2
5	A045E	Herbs, vegetables and oil sauces	1

NF: novel food.

#### 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 proposed use levels (Table 10), 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 10.

**Table 10:** Intake estimate 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)		P95 intake (mg/kg bw per day)	
		Lowest <sup>(a)</sup>	Highest <sup>(a)</sup>	Lowest <sup>(b)</sup>	Highest <sup>(b)</sup>
Infants	< 1	1	15	3	75
Young children <sup>(c)</sup>	1 to < 3	9	27	32	94
Other children	3 to < 10	5	24	23	73
Adolescents	10 to < 18	3	15	12	53
Adults <sup>(d)</sup>	≥ 18	3	9	16	38

bw: body weight.

(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 14/2/2022. 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 14/2/2022. 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): Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).

(d): Includes elderly, very elderly, pregnant and lactating women.

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).

### 3.7.4. Estimate of exposure to undesirable substances

Based on the P95 intake estimate (Table 10), EFSA calculated the exposure to undesirable substances (heavy metals, toxins, cyanide) for all population groups. The specification limits (Table 8) were used as maximum values for the concentration of 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. The Panel considers that the consumption of the NF under the proposed uses and use levels does not contribute substantially to the overall maximum safe intake of the analysed undesirable substances through diet. The assessment of the intake of manganese (Mn) from the NF, for which upper levels are not available, is provided in Section 3.9 Nutritional information.

The applicant indicated that cyanogenic glycosides (CNGs) may be present in the NF due to the use of cassava leaves as feed source. CNGs contain chemically bound cyanide (CN<sup>-</sup>) and are present in foods such as almonds, linseed or cassava; hence, residuals may be present in the insect gut. In all batches, the concentration of cyanide (CN<sup>-</sup>) in the NF is below the limit of detection of the analytical method used of 5 mg/kg. This value is lower than the mean upper bound content of total cyanide in other food groups, e.g. macaroons and amaretti 12.7 mg/kg, marzipan 8.4 mg/kg, grains for human consumption 6.4 mg/kg, and comparable to values reported in fruits products with cherries 4.6 mg/kg and biscuits (cookies) and 4.1 mg/kg (EFSA CONTAM Panel, 2019).

In 2016, the EFSA CONTAM Panel established an acute reference dose (ARfD) of 20 µg/kg bw for CN<sup>-</sup> in raw apricot kernels. In 2019, the EFSA CONTAM Panel concluded that the ARfD is applicable for acute effects of CN regardless the dietary source.

EFSA estimated the exposure to CN<sup>-</sup> from the NF using the specification limits of 5 mg/kg (Table 8), assuming complete CN<sup>-</sup> bioavailability, and the estimated daily intake of the NF. The highest chronic daily exposure to CN<sup>-</sup> from the NF was calculated for young children, resulting in 0.47 µg/kg bw (max P95), approximately 40 times lower than the ARfD. In other words, young children of 12 kg bw would need to consume 48 g of NF in order to reach the ARfD. Based on the chronic exposure assessment, the NDA Panel considers that it is unlikely that acute exposure would exceed the acute reference dose.

Based on the above, the Panel considers that any acute exposure to CN<sup>-</sup> from the NF is unlikely to reach the ARfD values. Therefore, content of CN<sup>-</sup> in the NF does not raise safety concerns.

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

No ADME data have been provided for the NF.

### 3.9. Nutritional information

The applicant provided nutritional analyses of the NF which consists mainly of protein, fat, dietary fibre (mainly chitin) and inorganic matter. The energy value of the NF is on average 360 kcal/100 g (Table 1). Analytical data on the amino acid composition, fatty acid content, minerals and vitamins in the NF have been provided for five independently produced batches of the NF.

The NF contains on average 75.3 g crude protein per 100 g, calculated using a protein-conversion factor of 6.25. The Panel notes that the use of the conventional factor overestimates the true protein content in the NF due to the presence of considerable amounts of non-protein nitrogen derived mainly from chitin (Janssen et al., 2017).

Boulos et al. (2020) determined an average conversion factor of 5.33 for whole *A. domesticus* as supported by literature data. Using this factor, the protein content of the NF amounts to 64.2 g/100 g, 17% lower than considering a conversion factor of 6.25. For regulatory purposes with respect to 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 the method FST-WI01 chapter 42 (Ref, EZ-FAAST) (LC-MS/MS). Results are reported in Appendix A in comparison to the recommended amino acid scoring pattern for children 6 months to 3 years and for older children, adolescents and adults (FAO, 2013).

The applicant investigated the protein digestibility of the NF by means of pepsin digestibility test (AOAC 971.09) on five batches of the NF. The test was conducted by an accredited laboratory in accordance with good laboratory practice (GLP). The digestibility was expressed as a percentage of the digestible protein over total crude protein, including non-protein nitrogen. As result, protein digestibility of the NF was  $83.2\% \pm 1.0\%$ . The applicant determined the protein quality as Protein Digestibility Corrected Amino Acid Score (PDCAAS) (FAO, 2011), using the recommended amino acid scoring patterns as reference values (FAO, 2013). The resulting PDCAAS value for the NF was 0.63 for children 6 months to 3 years with histidine being the most limiting amino acid followed by sulfur amino acids (methionine + cysteine) and leucine. For older children, adolescents and adults, the estimated PDCAAS was 0.79 with histidine as the limiting amino acid.

The Panel notes that protein quality as indicated by PDCAAS value for children 6 months to 3 years is relatively low. If the NF entirely replaces other protein sources of higher quality, it may negatively impact protein nutrition in case when overall protein intake is low. Based on the high (95th percentile) intake levels of the NF (Section 3.7.3, Table 10) with a maximum content of protein of 78% (Section 3.5, Table 7), the corresponding protein intake per kg bw per day from the NF would amount to 0.06 g for infants, 0.07 g for toddlers, 0.06 g for young children, 0.04 g for adolescents and 0.03 g for adults. These intakes correspond up to 4.5%, 7.6%, 6.7%, 4.9% and 3.5% of respective dietary reference values (DRVs) (EFSA NDA Panel, 2012) for protein for infants, toddlers, young children, adolescents and adults. Taking into account that the NF will not be the sole source of dietary protein, that it is integrated into a varied and mixed diet, and that the average protein intake in the EU population is high and frequently above DRVs (EFSA NDA Panel, 2012), the Panel considers that the consumption of the NF is not expected to negatively impact protein nutrition.

The major fatty acids in the NF are linoleic acid, palmitic acid, oleic acid and stearic acid (Appendix B). On average saturated, monosaturated fats and polyunsaturated fatty acids constitute 41.5%, 57.3% and 16.9% of total fatty acids, respectively. The content of trans fatty acids was below 0.05 g per 100 g NF.

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 the loss of catalytic efficacy. The NF contains on average 6.3 g chitin in 100 g (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), thereby possibly affecting their bioavailability, as reported for dietary fibres in general (Baye et al., 2017).

The applicant provided analytical data on the levels of some minerals and vitamins (Table 11).

**Table 11:** Content of micronutrients (minerals and vitamins) in the NF

Parameter	Batch number					Analytical method
	Colony1	Colony2	Colony3	Colony4	Colony5	
<b>Minerals (mg/100 g)</b>						
Sodium (mg/100 g)	438	435	456	320	330	AOAC 999.10:2005 (AAS)
Calcium (mg/100 g)	235	184	155	155	155	AOAC 927:02
Total phosphorous as P <sub>2</sub> O <sub>5</sub> (mg/100 g)	790	940	890	2160	2150	AOAC 995.11:2005
Magnesium (mg/100 g)	110	110	106	90	96	AOAC 2011.14 (ICP-OES)
Potassium (mg/100 g)	1094	1042	1161	939	1161	AOAC 999.10:2005 (AAS)
Iron (mg/100 g)	8.1	8.0	7.5	5.9	6.0	AOAC 999.10:2005 (modified AAS)
Manganese (mg/100 g)	10	9.0	9.6	8.9	9.2	AOAC 2011.14 (ICP-OES)
Copper (mg/100 g)	3.1	3.4	3.4	3.0	3.2	AOAC 2011.14 (ICP-OES)
Zinc (mg/100 g)	23	19	21	22	23	AOAC 2011.14 (ICP-OES)
Iodine (µg/100 g)	53	49	52	73	77	AOAC 2012.15
Selenium (µg/100 g)	60	120	20	–	20	AOAC 986.15 (ICP-OES)
Boron (mg/kg)	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	FDA EAM 4.4 V1-1 (ICP-OES)
Cobalt (mg/kg)	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	TCVN 9588:2013 (ICP-OES)
Nickel (mg/kg)	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	FDA EAM 4.4 V1-1 (ICP-OES)
<b>Vitamins (units)</b>						
Retinol (µg/100 g)	10.5	11.8	13.0	11.0	10.8	FST-WI01 chapter 31 (HPLC/DAD)
Thiamin (µg/100 g)	34	20	25	27	26	FST-WI01 chapter 60 (LC-MS/MS)
Cobalamin (µg/100 g)	< 100	< 100	< 100	< 100	< 100	CASE.SK.0124
Riboflavin (µg/100 g)	120	127	138	140	140	FST-WI01 chapter 60 (LC-MS/MS)
Alpha-tocopherol (mg/100 g)	2.6	2.2	2.3	2.4	2.5	AOAC 992.10:2005 (AAS)

AAS: atomic absorption spectrometry; ICP-OES: inductively coupled plasma optical emission; spectrometry; HPLC/DAD: high-performance liquid chromatography coupled with a diode-array detector; LC-MS: liquid chromatography-mass spectrometry; FST-WI Phương pháp do phòng thí nghiệm xây dựng/ Laboratory developed method; AOAC: Association of official analytical collaboration.

Considering the mean contents reported in Table 11 and the estimated P95th 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 group.

### Estimate daily intake of manganese

The concentration of Mn in the NF, according to specifications, may reach 100 mg/kg. This concentration is higher compared to food sources rich in Mn, e.g. nuts 24.9 mg/kg; dried fruit, nuts and seeds 11.9 mg/kg; chocolate 8.9 mg/kg; bread, miscellaneous cereals 8.0 mg/kg (EFSA NDA Panel, 2013). The Panel notes that the SCF (2000) reported that exposure to high levels of Mn by inhalation or oral intake may be neurotoxic. The SCF could, however, not set an UL for Mn and concluded that 'the margin between oral effect levels in humans as well as experimental animals and the estimated intake from food is very low. Given the findings on neurotoxicity and the potential higher susceptibility of some subgroups in the general population, oral exposure to Mn beyond the normally present in food and beverages could represent a risk of adverse health effects without evidence of any health benefit' (SCF/NDA, 2006).

EFSA estimated the intake of Mn from the NF, considering the product specification for Mn (Table 8) and the estimated daily intake of the NF for all population groups (Table 10). Results are presented in Table 12.



**Table 12:** Intake estimate of Mn 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 per day)		P95th intake (mg per day)	
		Lowest <sup>(a)</sup>	Highest <sup>(a)</sup>	Lowest <sup>(b)</sup>	Highest <sup>(b)</sup>
Infants	< 1	0.001	0.013	0.003	0.060
Young children <sup>(c)</sup>	1 to < 3	0.011	0.036	0.042	0.117
Other children	3 to < 10	0.010	0.060	0.041	0.166
Adolescents	10 to < 18	0.015	0.074	0.064	0.250
Adults <sup>(d)</sup>	≥ 18	0.026	0.068	0.117	0.241

Mn: manganese; NF: novel food.

(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 14/2/2022. 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 14/2/2022. 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): Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).

(d): Includes elderly, very elderly, pregnant and lactating women.

EFSA has previously reported that estimated mean Mn intakes for adults in the EU ranged from 2 to 6 mg/day, with the majority of values being around 3 mg/day (EFSA NDA Panel, 2013). In younger age groups, mean Mn intakes in various EU countries ranged from 1.5 to 3.5 mg/day in children, and from 2 to 6 mg/day in adolescents (EFSA NDA Panel, 2013).

The highest estimated mean intake of Mn from the NF across countries ranges between 0.01 mg/day in infants to 0.07 mg/day in adolescents. As compared to the highest mean background Mn intake estimates, the additional intake of manganese from the NF would be 1.0% for young children, 1.2% for adolescents and 1.1% in adults.

The highest estimated P95th intake of Mn from the NF ranges from 0.06 mg/day in infants to 0.25 mg/day in adolescents. As compared to the highest mean background Mn intake estimates, the additional intake of manganese from the NF would be 3.3% for young children, 4.2% for adolescents and 4.0% for adults.

The Panel considers that such an increase of Mn intake (< 5% of the highest mean background intake<sup>2</sup>) from the NF used as food ingredient is not of concern.

Insects may contain hydrogen cyanide, antinutritional factors (ANFs) such as tannins, oxalates, phytate (Jonathan, 2012; Shantibala et al., 2014), thiaminases (Nishimune et al., 2000) and protease inhibitors (Eguchi, 1993). The applicant determined the concentrations of total polyphenols, tannic acid, oxalic acid, hydrocyanic acid in five independently produced batches of the NF (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 13:** Levels of polyphenols and antinutrients in the NF

Parameter (unit)	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	Analytical method
Total polyphenols (as gallic acid) g(100g)	0.78	0.78	0.74	0.76	0.80	AOAC 2017.13
Tannic acid (%)	1.25	1.25	1.17	1.21	1.22	ISO 14502-1:2005
Oxalic acid (mg/kg)	126	152	437	427	220	HPLC-DAD
Hydrocyanic acid (mg/kg)	< 5	< 5	< 5	< 5	< 5	915.03:2005

AOAC: association of official analytical collaboration; HPLC: high-performance liquid chromatography; DAD: diode-array detection.

<sup>2</sup> As reported in the published minutes of the 131st meeting of the working group on novel foods (WG NF 2022), the working group (WG) considered that for the purpose of the assessment of NFs, intakes that lead to a significant increase of Mn intake as compared to the background diet are considered of concern. The WG also noted that an assessment of UL for Mn is ongoing (EFSA-Q-2021-00371). Based on experts' judgement and criteria set by the WHO/FAO's Codex Alimentarius Commission (2015) for selecting foods/food groups that contribute significantly to total dietary exposure of a contaminant or toxin, the WG concluded that Mn intake above 5% as compared to the high mean background intake (EFSA NDA Panel 2013) is considered as a significant contribution.

Analytical data of the nitrate and nitrite concentration for five independently produced batches of the NF have been provided (FST-WI 08 chapter 88 (IC-CD)). Values of nitrite were below limit of detection of 10 mg/kg. Values of nitrate were between 24.4 and 33.6 mg/kg. Considering the mean content (29.7 mg/kg) and the estimated P95 of exposure to the NF, the Panel notes that the intake of nitrate from the NF is below the current acceptable daily intake (ADI) of 3.7 mg/kg bw/day (EFSA ANS Panel, 2017).

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 applicant performed a literature search and no papers were found concerning the toxicology of *A. domesticus*. No toxicological studies were conducted with the NF under assessment.

The toxicological profile of the source of the NF *A. domesticus* has been previously assessed by the Panel (EFSA NDA Panel, 2021). The Panel concluded that no safety concerns arose from the history of use of *A. domesticus*.

The Panel notes that the NF under assessment can be considered representative of the previously assessed *A. domesticus* (EFSA NDA Panel, 2021) only with regard to the profile of endogenously produced compounds of possible concerns, but not for compositional data and for any compound that can be present due to rearing condition (e.g. feed) or processing. The Panel notes that no concerns could be identified based on compositional data of the NF and production process.

Regarding the safety of chitin present in the NF, the applicant referred to the EFSA's scientific opinion on the safety of 'chitin-glucan' as a NF ingredient (EFSA NDA Panel, 2010). However, the Panel is of the view that the polymer chitin-glucan cannot be considered as representative of the chitin derived from *A. domesticus*. 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 (equivalent to 4,500 mg/kg bw per day<sup>3</sup>). No firm conclusions could be drawn from the study by the Panel since only the abstract was accessible (Niho et al., 1999).

#### 3.10.1. Human data

The applicant did not provide any human studies conducted with the NF or its source. No human studies were retrieved from the literature search.

### 3.11. Allergenicity

The Panel has previously considered that the consumption of the NF source (*A. domesticus*) might induce primary sensitisation to *A. domesticus* proteins. The Panel also considered that allergic reactions may occur in subjects allergic to crustaceans, mites and molluscs (cross-reactivity) (EFSA NDA Panel, 2021).

In regard to chitin, it 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) after intranasal or intraperitoneal administration and this implies the potential to promote hypersensitivity (Komi et al., 2018).

From literature research, the Panel has noted that additional allergens may end up in the NF, if these allergens are present in the substrate fed to the insects. This may include allergens listed in the Annex II of Regulation (EU) No 1169/2011 (2011) (EFSA NDA Panel, 2021).

## 4. Discussion

The NF which is subject of the application is partially defatted house cricket (*A. domesticus*), dried in the form of powder. 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. The concentration of contaminants in the NF depends 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

<sup>3</sup> Considering the conversion factor of 0.09 proposed by the EFSA Scientific Committee (2012) for subchronic rat studies.

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. The highest intake estimate per kg bw basis was calculated for young children (1–< 3 years old) at 94 mg NF/kg bw per day at the 95th percentile of the intake distribution.

CN<sup>-</sup> may be present in the NF if cassava parts are used as feed. The Panel considers that any acute exposure to CN<sup>-</sup> from the NF is unlikely to reach the ARfD values if the specifications and proposed uses and use levels are met. Therefore, content of CN<sup>-</sup> in the NF does not raise safety concerns.

The Panel notes that consumption of the NF under the proposed uses and use levels does not contribute substantially to the total dietary exposure of analysed undesirable substances (heavy metals, toxins, cyanide).

The Panel notes that NF has a high protein content with a relatively low PDCAAS value of 0.63 for children 6 months to 3 years with histidine as the most limiting amino acid followed by sulfur amino acids and leucine. The Panel notes the true protein levels in the NF are overestimated due to the presence of non-protein nitrogen of chitin. Considering that the NF will not be the sole source of dietary protein and is integrated into a varied and mixed diet, the consumption of the NF is not expected to negatively impact protein nutrition.

None of the existing upper levels of the analysed micronutrients are expected to be exceeded considering the proposed uses and use levels. The NF used as a food ingredient was estimated to increase the high mean background intake of Mn by less than 5%, and was therefore not considered of concern.

The reported concentrations of the antinutritional factors in the NF are comparable to those in other foods. 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. Taking into account the composition of the NF and the proposed conditions of use, the Panel concludes that the consumption of the NF is not nutritionally disadvantageous. The Panel notes that no genotoxicity and no subchronic toxicity studies were provided by the applicant. No safety concerns arise from the history of use of *A. domesticus* or from the compositional data of the NF. Given that the NF under assessment can be only considered representative of the previously assessed *A. domesticus* (EFSA NDA Panel, 2021) with regards to the profile of endogenously produced compounds of possible concerns, the Panel identified no other safety concerns than allergenicity.

The Panel considers that the consumption of the NF might trigger primary sensitisation to *A. domesticus* protein. 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) might 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 may 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 detailed description of the production process, proximate analysis, contaminants analyses, stability studies, microbiological analysis, digestibility of protein study report claimed proprietary by the applicant.

## 6. Recommendation

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

## 7. Steps taken by EFSA

- 1) On 08/07/2020 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of defatted whole cricket (*Acheta domesticus*) powder as a novel food Ref. Ares (2020)3602284.

- 2) On 08/07/2020, a valid application on defatted whole cricket (*Acheta domesticus*) powder, which was submitted by Cricket One Co. Ltd, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2019/1227) and the scientific evaluation procedure was initiated.
- 3) On 23/10/2020, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
- 4) On 01/07/2021, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
- 5) On 22/07/2021, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
- 6) On 01/10/2021, EFSA received a letter from the European Commission with the revised request for a scientific opinion on defatted house cricket (*Acheta domesticus*) powder as a novel food Ref. Ares (2021)5980800.
- 7) On 08/12/2021, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
- 8) On 01/02/2022, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended;
- 9) On 24/02/2022, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
- 10) During its meeting on 23/03/2022, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of defatted house cricket (*Acheta domesticus*) powder as a NF pursuant to Regulation (EU) 2015/2283.

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## Abbreviations

3MCPD	3-monochloropropane-1,2-diol or 3-chloropropane-1,2-diol
μECD	microelectron capture detection
AAS	atomic absorption spectrometry
ADF	acid detergent fibre
ADI	acceptable daily intake
ADL	acid detergent lignin
ADME	absorption, distribution, metabolism and excretion
AMCase	acidic mammalian chitinase
ANFs	antinutritional factors
ANS	Panel on Food Additives and Nutrient Sources added to Food
AOAC	association of official analytical collaboration
ARfD	acute reference dose
BIOHAZ	EFSA Panel on Biological Hazards
bw	body weight
CASE.SK	Center for Analysis Service of Experiment of HCMC
cfu	colony forming units

CN <sup>-</sup>	Cyanide
CNGs	cyanogenic glycosides
CONTAM	EFSA Panel on Contaminants in the Food Chain
DIN	Deutsches Institut für Normung
DRVs	dietary reference values
EN	Europäische Norm
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization
FSANZ	Food Standards Australia New Zealand
FST-WI	Phương pháp do phòng thí nghiệm xây dựng/ Laboratory developed method
GC/ $\mu$ ECD	gas chromatography with microelectron capture detector
GC-MS	gas chromatography-mass spectrometry
GC-MS/MS	gas chromatography coupled mass spectrometry
GMO	Genetically modified organism
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Points
HPLC	high-performance liquid chromatography
HPLC/DAD	high-performance liquid chromatography coupled with a diode-array detector
HRGC	high-resolution gas chromatography
HRMS	high-resolution mass spectrometry
IAC	immunoaffinity column cleanup
ICP-OES	inductively coupled plasma optical emission
IL	Interleukin
ISO	International Organization for Standardization
LC-MS	liquid chromatography-mass spectrometry
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LOQ	limits of quantification
meq	milliequivalents
MUFA	monounsaturated fatty acids
ND	not detected
NDA	Panel on Nutrition, Novel Foods and Food Allergens
NF	novel food
PCB	polychlorinated biphenyl
PDCAAS	protein digestibility corrected amino acid score
PP	polypropylene
PUFA	polyunsaturated fatty acids
RH	relative humidity
RPLC/FD	reversed-phase liquid chromatography/fluorescence detector
SCF	Scientific Committee on Food
TAMC	total aerobic microbial count
TVCN	Vietnam Standards
TYMC	total yeast and mould count
UB	upper bound
UCT	University of Chemistry and Technology (Prague)
WHO (2005) PCDD/F-TEQ	sum of polychlorinated dibenzo- <i>p</i> -dioxins-polychlorinated dibenzofurans-polychlorinated biphenyls expressed as World Health Organization toxic equivalent
w/w	weight per weight

## Appendix A – Batch to batch amino acid analysis of the NF

Amino acids (mg/g total amino acids)	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	Average	FAO/WHO 2013 <sup>(a)</sup>	FAO/WHO 2013 <sup>(b)</sup>
<b>Essential</b>								
Histidine	17.0	12.6	11.4	18.8	16.0	15.2	20	16
Isoleucine	66.9	63.6	63.3	45.2	49.2	57.6	32	30
Leucine	69.4	58.1	66.9	50.8	45.3	58.1	66	61
Lysine	64.7	64.7	70.4	63.6	61.5	65.0	57	48
Methionine	16.0	16.6	17.0	16.1	15.6	16.3	27	23 <sup>(c)</sup>
Phenylalanine	37.7	32.9	34.4	35.1	38.0	35.6	52	41 <sup>(d)</sup>
Threonine	40.0	41.7	44.7	48.3	51.7	45.3	31	25
Tryptophan	10.0	9.9	9.0	14.1	14.1	11.4	8.5	6.6
Valine	71.9	73.9	42.5	85.9	88.9	72.6	43	40
<b>Conditionally essential</b>								
Arginine	85.6	85.6	82.8	61.5	59.2	74.9		
Cystine	4.4	6.4	4.9	8.8	9.1	6.7		
Glycine	56.5	56.5	65.3	72.8	81.8	66.6		
Proline	57.1	53.5	63.1	73.5	78.8	65.2		
Tyrosine	60.0	81.1	64.2	44.7	40.6	58.1		
<b>Non-essential</b>								
Alanine	87.4	96.9	95.6	95.5	101.3	95.4		
Aspartic acid	81.9	77.1	90.7	109.5	98.3	91.5		
Glutamic acid	114.1	103.8	109.5	134.7	128.1	118.1		
Serine	59.3	64.8	64.4	21.1	22.6	46.4		

(a): Recommended amino acid scoring patterns for children (6 months to 3 years old).

(b): Recommended amino acid scoring patterns for other child, adolescents and adults.

(c): Methionine + cysteine.

(d): Phenylalanine + tyrosine.



## Appendix B – Major fatty acids in the NF

Fatty acids (% of total fatty acids)	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5
Linoleic acid	37.3	36.4	36.7	39.4	40.3
Palmitic acid	31.1	31.5	31.5	25.3	26.0
Oleic acid	17.1	17.3	17.2	14.8	14.8
Stearic acid	9.4	9.6	9.5	12.7	11.8
Gamma linolenic acid	1.2	1.1	1.2	–	–
Saturated fatty acid	42.2	42.8	42.6	40.2	39.8
MUFA	17.6	17.8	17.8	15.6	15.6
PUFA	39.7	38.8	39.0	41.8	42.4
Trans fatty acid	–	–	–	–	–
Omega 3	0.9	0.9	0.9	1.6	1.4
Omega 6	38.7	37.8	38.1	40.0	40.9

MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.  
Analytical method: AOAC 996,06:2011.

## **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/full/10.2903/j.efsa.2022.7258> under supporting information <https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2022#support-information-section>).