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Safety of frozen and dried formulations from whole house crickets (*Acheta domesticus*) 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 house crickets (*Acheta domesticus*) as a novel food pursuant to Regulation (EU) 2015/2283. The NF is proposed in three formulations: (i) frozen, (ii) dried, (iii) ground. 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 concentrations of contaminants in the NF depend 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 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 in the form of a 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 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 *A. domesticus* or from the compositional data of the 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. The Panel concludes that the NF is safe under the proposed uses and use levels.

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1. Introduction

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

On 17 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 authorise placing on the market of whole and ground crickets (*A. domesticus*) as a novel food (NF).

The application requests to authorise use of whole and ground crickets (*A. domesticus*) in a number of foods. The target population is the general population above the age of 3 years. 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 crickets (*A. domesticus*) as a novel food.

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 "cricket" is generic and does not exclusively refer to *A. domesticus* and therefore it should be replaced by "house cricket". The term "whole and ground" indirectly implies that *A. domesticus* powder may not be a product derived from using the whole insect. Moreover, the wording "frozen and dried formulations" would be a more inclusive descriptor for all three formulations of the novel food. On that basis, the Commission amended the title to "Revised request for a scientific opinion on frozen and dried formulations from whole house crickets (*Acheta domesticus*) 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 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) 2017/2469¹.

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, 2016a). 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 *A. domesticus* sp. (house crickets). The NF falls under the category '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 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, soups and meat products. Products with the NF can be consumed by the general population.

3.2. Identity of the NF

The NF consists of frozen, dried and ground formulations of adult house crickets. The term 'house cricket' refers to *A. domesticus*, an insect species that belongs to the family of Gryllidae, Subfamily Gryllinae, Genus *Acheta*. *A. domesticus* is present in various regions worldwide, including Australia, Asia, Africa, Europe and North America (GBIF, 2017). 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.

The NF is intended to be marketed as (A) blanched and frozen *A. domesticus* (AD frozen); (B) blanched and freeze-dried *A. domesticus* (AD dried); (C) blanched, freeze-dried and ground *A. domesticus* (AD 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 Good Manufacturing Practice (GMP) and 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 distinctive parts, i.e. farming, harvesting and post-harvest processing. All steps take place in a closed farming system.

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. The applicant demonstrated that plastic components are not ingested by the insects and reported that no pesticides, antimicrobials, anti-parasitic agents or solvents are used during the entire production process.

The applicant reported that the feed used to feed *A. domesticus* is a plant-derived material compliant with Directive 2002/32/EC² and produced according to GMP+.

During farming *A. domesticus* can be affected by pathogens including the cricket paralysis virus (CrPV) of the Dicistroviridae family, the cricket densovirus (AdDV) from the Parvoviridae family (Szelei et al., 2011; Maciel-Vergara and Ros, 2017), Iridovirus CrIV (Kleespies et al., 1999), *Penaeus merguensis* densovirus (Pmerg DNV) (La Fauce and Owens, 2008; Owens et al., 2011) and the nematode *Heterorhabditis Georgiana* (Shapiro-Ilan et al., 2009). Literature review conducted by the applicant highlighted that these pathogens are specific to insects, and non-pathogenic for humans or

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.

other vertebrates. Examples of food-borne bacteria that may be present in *A. domesticus* include *Citrobacter*, *Klebsiella* and *Yersinia* (Ulrich et al., 1981). However, their potential presence in the NF is monitored by microbiological analysis of *Enterobacteriaceae* as reported in Section 3.4, Table 4.

Adult insects are harvested (4–5 weeks old) after being separated from the substrate and faeces. Decayed insects are removed after visual inspection. After the harvest, a 24-h fasting step is implemented to allow the insects to discard their bowel content.

The post-harvest processing includes killing of the adults by freezing and storing at -18°C . Three formulations of the NF are then obtained by processing using the whole insect. The formulation 'AD frozen' is obtained by rinsing in water, blanching in hot water ($> 90^{\circ}\text{C}$ for at least 10 min) and freezing. The formulation 'AD dried' is obtained by rinsing in water, blanching in hot water ($> 90^{\circ}\text{C}$ for at least 10 min) and freeze-drying. The formulation 'AD powder' is obtained after rinsing in water, blanching in hot water ($> 90^{\circ}\text{C}$ for at least 10 min), freeze-drying and grinding.

The thermal treatment contributes to the reduction of the microbial load of the insects as well as the elimination of potentially present viruses and parasites, and reduction of enzymatic activity. Dehydration of the insects takes place in freeze dryers, resulting in a final product with moisture $< 5\%$. The AD powder is obtained via mechanical grinding of the insect and sieving to reduce particle size below < 1 mm. The formulations of the NF are stored in sealed packaging at -18°C (AD frozen) or at room temperature (AD dried, AD 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 different batches of the NF formulations i.e. (a) AD frozen; (b) AD dried; (c) AD powder. For all parameters, at least five independently produced batches were analysed. Based on the description of the production process, the Panel considers the two formulations of the NF (AD frozen and AD dried) are representative of each other regarding most of their compositional parameters, when the difference in moisture is taken into account. The Panel also considers the proximate composition of AD dried as representative of AD powder. The representativeness is in all cases excluded for microbiological parameters.

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. Whenever in-house methods were employed, a full description of the method as well as results of the respective validation procedures has been provided.

It should be noted that 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, 2016a).

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 AD frozen and AD dried

AD frozen						Analytical method
Parameter (unit)	Batch number					
	#1	#2	#3	#4	#5	
Crude protein (g/100 g of NF)	14.8	14.7	15.6	15.7	14.7	Kjeldahl ($N \times 6.25$)
Fat (g/100 g of NF)	5.8	5.7	6.2	5.8	5.8	EC-152/2009 (gravimetry)
Digestible carbohydrate (g/100 g of NF)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	Titrimetry (Luff School)
Dietary fibre (g/100 g of NF)	1.1	1.1	1.1	1.2	1.3	AOAC 2009.01
Sugars (g/100 g of NF)	$< \text{LOQ}$	$< \text{LOQ}$	$< \text{LOQ}$	$< \text{LOQ}$	$< \text{LOQ}$	NEN-3571; EC-152/2009
Ash (g/100 g of NF)	0.7	0.7	0.6	0.6	0.7	EC-152/2009
Moisture (g/100 g of NF)	78.8	78.9	78.3	77.8	77.9	Gravimetric method
Energy value (kJ/100 g)	480	470	500	490	470	Regulation (EU) No 1169/2011

AD dried						
Parameter (unit)	Batch number					Analytical method
	#6	#7	#8	#9	#10	
Crude protein (g/100 g of NF)	59.5	60.8	60.1	60.4	60.5	Kjeldahl, (N × 6.25)
Fat (g/100 g of NF)	31.5	30.7	31.5	31.8	30.9	EC-152/2009
Digestible carbohydrate (g/100 g of NF)	2.1	2.0	2.2	2.1	2.2	Titrimetry (Luff Schoorl)
Dietary fibre (g/100 g of NF)	4.0	3.9	4.2	4.4	4.7	AOAC 2009.01
Sugars (g/100 g of NF)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	NEN-3571; EC-152/2009
Ash (g/100 g of NF)	3.0	3.0	3.0	3.1	3.1	EC-152/2009
Moisture (g/100 g of NF)	0.6	0.6	0.7	1.5	0.7	Gravimetric method
Energy value (kJ/100 g)	2,200	2,200	2,300	2,300	2,200	Regulation (EU) 1169/2011

AOAC: Association of Official Analytical Chemists.

The Panel notes the values for some proximate parameters vary between batches, but this can be expected due to variability inherent to the analytical methods and individual whole insect composition. The values may also depend on the rearing conditions (feed, developmental stage at the time of harvesting, ambient conditions) (Rumpold and Schlüter, 2013a; Oonincx et al., 2015).

Regarding the crude protein content of the NF, the Panel notes that literature (Janssen et al., 2017) suggests 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 the section '3.9 Nutritional information'.

Chitin 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). 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 5 independently produced batches in AD dried (Table 2). 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 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 in AD dried

Parameter (g/100 g NF)	Batch number				
	#6	#7	#8	#9	#10
ADF	8.3	7.9	8.0	8.5	8.1
ADL	< 1.5	1.8	< 1.5	1.7	1.5
Chitin	8.3 ^(a)	6.1	8.0 ^(a)	6.8	6.6

ADF: Acid Detergent Fibre; ADL: Acid Detergent Lignin, LOQ: 1.5; Chitin calculated as ADF-ADL.

(a): The results may present a small overestimation of chitin, due to the uncertainty regarding the ADL numerical value.

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

Upon EFSA's request, the applicant also undertook speciation analysis and determined the content of inorganic arsenic in the NF, which was found to be < 0.02 mg/kg in five independently produced batches of AD dried.

Table 3: Heavy metals, mycotoxins and dioxins levels in AD dried

Parameter	Analytical method	#6	#7	#8	#9	#10
Heavy metals (mg/kg)						
Arsenic (As)	Internal method, ICP-MS	0.90	0.96	0.90	0.92	0.93
Mercury (Hg)		0.038	0.041	0.037	0.038	0.039
Lead (Pb)		< 0.02	0.05	< 0.02	< 0.02	< 0.02
Cadmium (Cd)		0.06	0.06	0.06	0.06	0.06
Mycotoxins (µg/kg)						
Aflatoxins B1	Internal Method, IAC-LC-FLD	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Aflatoxins B2		< 0.04	< 0.04	< 0.04	< 0.04	< 0.04
Aflatoxins G1		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Aflatoxins G2		< 0.06	< 0.06	< 0.06	< 0.06	< 0.06
Aflatoxins (Sum of B1, B2, G1, G2)		< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Ochratoxin A	Internal Method, IAC-LC-FLD	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4
Nivalenol	Internal Method, LC-MS/MS	< 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	< 0.012	< 0.012	< 0.012	< 0.012	< 0.012
Fumonisin B2		< 0.0049	< 0.0049	< 0.0049	< 0.0049	< 0.0049
Dioxins (pg/g fat)						
Sum of dioxins and dl-PCBs (UB, WHO-TEQ ₂₀₀₅)	EC 2017/644, GC-MS/MS	1.23	1.38	1.27	1.41	1.24

ICP-MS: Inductively Coupled Plasma Mass Spectrometry; IAC-LC-FLD: Immunoaffinity Chromatography-Liquid Chromatography Fluorescence Detector; LC-MS/MS: Liquid Chromatography-tandem Mass Spectrometry; GC-MS/MS: Gas Chromatography-tandem Mass Spectrometry; UB: Upper Bound; Sum of dioxins and dl-PCBs (WHO-TEQ₂₀₀₅) = sum of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls by using toxicity equivalence factors (TEF) set by World Health Organization in 2005.

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 AD powder are below the limits of quantification (LOQ) of the implemented methods (GC-MS ITD Equal CEN/TR 16468 and LC-MS Equal CEN/TR 15641) with the exception of piperonyl butoxide for which concentrations are 0.01 mg/kg.

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 AD dried. Average histamine levels were 2.96 and 1.88 mg/kg at $t = 0$ and $t = 12$ months, respectively. High concentration levels of spermidine (150 mg/kg and 115 mg/kg at $t = 0$ and $t = 12$ months, respectively) and spermine (25 mg/kg and 245 mg/kg at $t = 0$ and $t = 12$ months, respectively) were reported. No legal maximum levels have been established for spermidine and spermine in foods. Similar concentrations have been reported in legumes (206 mg/kg and 68.82 mg/kg, respectively), cereals (353 mg/kg and 145.84 mg/kg, respectively) and fresh meat (13 mg/kg and 69 mg/kg, respectively) (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 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 analysed the NF for *Pseudomonas aeruginosa* which belonging to *Pseudomonas* genus could have been contributed to the occurrence of biogenic amines in the NF. For all forms of the NF, *P. aeruginosa* was reported at levels < 10 cfu/g.

The applicant provided microbiological data on five independently produced batches of all NF formulations (AD frozen, AD dried, AD powder) (Table 4). The Panel notes that in some cases the applicant did not provide the actual values for total aerobic count, but instead the quantification limits

as defined by the dilutions used upon the analyses. However, 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

AD frozen			Batch number				
Parameter	Unit	Method	#1	#2	#3	#4	#5
Total aerobic count	cfu/g	Equivalent to ISO 4833	< 1,000	< 1,000	< 1,000	< 1,000	< 1,000
<i>Enterobacteriaceae</i>	cfu/g	Equivalent to NEN-ISO 21528-2	< 10	< 10	< 10	< 10	< 10
<i>Escherichia coli</i>	cfu/g	Plate Counting Method ISO:16649-2:2001	< 10	< 10	< 10	< 10	< 10
<i>Listeria monocytogenes</i>	In 25 g	Equivalent to NEN-EN-ISO 11290-1	ND	ND	ND	ND	ND
<i>Salmonella</i> spp.	In 25 g	Equivalent to ISO 6579	ND	ND	ND	ND	ND
<i>Bacillus cereus</i>	cfu/g	Equivalent to ISO 7932	< 10	< 10	< 10	< 10	< 10
Coagulase positive staphylococci	cfu/g	Equivalent to NEN-EN-ISO 6888-2, 37°C	<10	<10	<10	<10	<10
<i>Clostridium perfringens</i>	cfu/g	Equivalent to ISO 7937	< 10	< 10	< 10	< 10	< 10
<i>Campylobacter</i> spp.	In 25 g	NEN-EN-ISO 10272-1	ND	ND	ND	ND	ND
Yeast and Moulds	cfu/g	Equivalent to ISO 7954:1987	< 10	< 10	< 10	< 10	< 10
AD dried			Batch number				
Parameter	Unit	Method	#6	#7	#8	#9	#10
Total aerobic count	cfu/g	Equivalent to ISO 4833	< 4,000	< 1,000	< 1,000	< 1,000	< 1,000
<i>Enterobacteriaceae</i>	cfu/g	Equivalent to NEN-ISO 21528-2	< 10	< 10	< 10	< 10	< 10
<i>Escherichia coli</i>	cfu/g	Plate Counting Method ISO:16649-2:2001	< 10	< 10	< 10	< 10	< 10
<i>Listeria monocytogenes</i>	In 25 g	equivalent to NEN-EN-ISO 11290-1	ND	ND	ND	ND	ND
<i>Salmonella</i> spp.	In 25 g	Equivalent to ISO 6579	ND	ND	ND	ND	ND
<i>Bacillus cereus</i>	cfu/g	Equivalent to ISO 7932	< 10	< 10	< 10	< 10	< 10
Coagulase positive staphylococci	cfu/g	Equivalent to NEN-EN-ISO 6888-2, 37°C	< 10	< 10	< 10	< 10	< 10
<i>Clostridium perfringens</i>	cfu/g	Equivalent to ISO 7937	< 10	< 10	< 10	< 10	< 10
<i>Campylobacter</i> spp.	In 25 g	NEN-EN-ISO 10272-1	ND	ND	ND	ND	ND
Yeast and Moulds	Cfu/g	Equivalent to ISO 7954:1987	< 10	< 10	< 10	< 10	< 10
AD powder			Batch number				
Parameter	Unit	Method	#11	#12	#13	#14	#15
Total aerobic count	cfu/g	Equivalent to ISO 4833	< 10,000	14,000	14,000	19,000	26,000
<i>Enterobacteriaceae</i>	cfu/g	Equivalent to NEN-ISO 21528-2	< 10	< 10	< 10	< 10	< 10
<i>Escherichia coli</i>	cfu/g	Plate Counting Method ISO:16649-2:2001	< 10	< 10	< 10	< 10	< 10
<i>Listeria monocytogenes</i>	In 25 g	Equivalent to NEN-EN-ISO 11290-1	ND	ND	ND	ND	ND
<i>Salmonella</i> spp.	In 25 g	equivalent to ISO 6579	ND	ND	ND	ND	ND
<i>Bacillus cereus</i>	cfu/g	Equivalent to ISO 7932	< 10	< 10	< 10	< 10	< 10

Coagulase positive staphylococci	cfu/g	Equivalent to NEN-EN-ISO 6888-2, 37°C	< 10	< 10	< 10	< 10	< 10
<i>Clostridium perfringens</i>	cfu/g	Equivalent to ISO 7937	< 10	< 10	< 10	< 10	< 10
<i>Campylobacter</i> spp.	In 25 g	NEN-EN-ISO 10272-1	ND	ND	ND	ND	ND
Yeast and Moulds	cfu/g	Equivalent to ISO 7954:1987	140	< 40	< 10	< 40	< 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 AD frozen, AD dried, and upon request for AD powder (Table 5). The NF forms have been analysed immediately after manufacturing (0 months) and after storage at room temperature (AD dried and AD powder) or at -18°C (AD frozen) for 12 months. Microbiological data at 3, 6 and 9 months were also provided for AD frozen and AD dried, and at 6 months for AD powder.

The applicant did not provide the actual values of the total aerobic count, but instead the quantification limits as defined by the dilutions used. The Panel notes that the microbiological values of most of the analysed samples do not exceed the given specification limits.

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

AD frozen		0 months					12 months				
Parameter	Unit	#1	#2	#3	#4	#5	#16	#17	#18	#19	#20
Total aerobic count	cfu/g	< 1,000	< 1,000	< 1,000	< 1,000	< 1,000	< 1,000	< 1,000	< 1,000	< 1,000	< 1,000
<i>Enterobacteriaceae</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
<i>Escherichia coli</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
<i>Listeria monocytogenes</i>	In 25 g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Salmonella</i> spp.	In 25 g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Bacillus cereus</i>	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
<i>Clostridium perfringens</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
<i>Campylobacter</i> spp.	In 25 g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Yeast and Moulds	cfu/g	< 10	< 10	< 10	< 10	< 10	< 40	< 10	< 10	< 10	< 40
AD dried		0 months					12 months				
Parameter	Unit	#6	#7	#8	#9	#10	#21	#22	#23	#24	#25
Total aerobic count	cfu/g	< 4,000	< 1,000	< 1,000	< 1,000	< 1,000	< 10	< 10	< 10	< 10	< 10
<i>Enterobacteriaceae</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
<i>Escherichia coli</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
<i>Listeria monocytogenes</i>	In 25 g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Salmonella</i> spp.	In 25 g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Bacillus cereus</i>	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
<i>Clostridium perfringens</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
<i>Campylobacter</i> spp.	In 25 g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Yeast and Moulds	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10

AD powder	Unit	0 months					12 months				
		#11	#12	#13	#14	#15	#11	#12	#13	#14	#15
Total aerobic count	cfu/g	< 1,000	14,000	14,000	19,000	26,000	21,000	< 4,000	24,000	< 1,000,000	18,000
<i>Enterobacteriaceae</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
<i>Escherichia coli</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
<i>Listeria monocytogenes</i>	In 25 g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Salmonella</i> spp.	In 25 g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Bacillus cereus</i>	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
<i>Clostridium perfringens</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
<i>Campylobacter</i> spp.	In 25 g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Yeast and Moulds	cfu/g	140	< 40	< 10	< 40	< 10	< 100	< 40	< 100	< 100	< 40

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 (AD powder) (Table 6). Peroxide value (PV), p-anisidine (PA) and free fatty acids (FFA) have been determined. The data provided cover a period of at least 12 months, which is the proposed shelf-life.

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

Time (months)	Analytical method	Batch number									
		0					12				
Parameter (unit)		#11	#12	#13	#14	#15	#11	#12	#13	#14	#15
Water activity	NEN-EN-ISO 18787:2017	0.458	0.456	0.492	0.457	0.456	0.451	0.450	0.453	0.462	0.452
Free fatty acids (expressed as % oleic acid of total fat)	NEN-EN-ISO 660:2009	1.9	1.9	1.5	1.5	1.8	1.2	2.4	2.0	2.2	2.1
Peroxide value (meq O ₂ /kg fat)	NEN-EN-ISO 3960:2010	1.6	3.7	2.4	2.4	1.6	1.5	4.3	3.2	3.1	3.6
p-Anisidine value	NEN-EN-ISO 6885:2000	1.3	< 1.0	< 1.0	< 1.0	2.6	< 1.0	2.3	4.2	1.5	1.3

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 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 the microbiological stability of 'cricket burger', 'cricket snack' and 'cricket-based falafel premix'. Data were provided from five independently produced batches of each product stored at -18°C at $t = 0$ and $t = 6$ months (shelf-life of these products is 6 months). A sensory panel analysis was performed for all products and off-flavours were not reported.

The cricket burger was a mixture of AD powder (30%), vegetables, sunflower oil, egg and spices, which went through a thermal processing (140°C for 30 s) and a blast freezing step. It had average water activity of 0.944. The content of process contaminants has been provided at $t = 0$. Furan levels of $< 5 \mu\text{g}/\text{kg}$ and free 3-MCPD levels of $< 10 \mu\text{g}/\text{kg}$ were reported. FFA expressed as proportion of oleic acid in total fat were reported to be in the range of 0.4–1.2% to 0.5–1.0% in the product at $t = 0$ and $t = 6$ months, respectively. Peroxide values of the product were up to 5.5 meq/kg fat (mean

4.5 meq/kg fat) at $t = 0$ and up to 4.4 meq/kg fat (mean 2.1 meq/kg fat) at $t = 6$ months. High values of *p*-anisidine were found in the product, ranging from 30.3 to 48.5 (mean 38.1) at $t = 0$ to 20.6–38.4 (mean 28.7) at $t = 6$ months. According to the applicant, the high *p*-anisidine value of the product might be due to the frying oil used during food production to pre-cook the burger as suggested by Johansson et al. (1995).

The cricket snack was a ready-to-eat product made of the AD dried (25%) mixed for 15 minutes at 70°C with roasted chickpeas, chickpeas flour, caramelised sugar and spices. It had average water activity of 0.426. The content of process contaminants has been provided at $t = 0$. Furan levels up to 6.9 µg/kg and polycyclic aromatic hydrocarbons (PAH4) levels of 2.2 µg/kg (lower bound) were reported. FFA expressed as proportion of oleic acid in total fat were reported to be in the range of 4.9–6.8% to 3.7–5.5% in the product at $t = 0$ and $t = 6$ months, respectively. Peroxide values of the product were up to 81.1 meq/kg fat (mean 62.2 meq/kg fat) at $t = 0$ and up to 93.3 meq/kg fat (mean 76.8 meq/kg fat) at $t = 6$ months. The *p*-Anisidine value ranged from 8.4 to 10.5 (mean 9.2) at $t = 0$ to 6.9–13.1 (mean 9.2) at $t = 6$ months. The applicant assumed that other ingredients and roasting the product at high temperature could have led to such high peroxide and *p*-anisidine values.

The applicant provided data for a 'falafel premix' powder product containing AD powder (30%), grain flours and spices (mixed at 40–50°C for 10 min). It had average water activity of 0.323. The content of process contaminants has been provided at $t = 0$. Furan levels < 5 µg/kg and PAH levels < 2.0 µg/kg were reported. FFA expressed as proportion of oleic acid in total fat were reported to be in the range of 2.0–2.2% to 2.1–2.6% in the product at $t = 0$ and $t = 6$ months, respectively. Peroxide values of the product were 1.04 meq/kg fat (mean value) at $t = 0$ and 0.94 meq/kg fat (mean value) at $t = 6$ months. The mean *p*-anisidine value was 1.7 at $t = 0$ and 6.7 at $t = 6$ months.

The Panel notes the limited number of analysed samples and the absence of control samples and considers that no conclusions can be made regarding the stability of the NF when used as ingredient in other foodstuffs.

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 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 processing-generated contaminants and microbiological criteria, the stability data do not raise safety concerns.

3.5. Specifications

The specifications of the NF are indicated in Table 7.

Table 7: Specifications of the NF

Parameter	Unit	AD frozen	AD dried; AD powder
Description:			
AD frozen: thermally processed, frozen, whole <i>A. domesticus</i>			
AD dried: thermally processed, dried, whole <i>A. domesticus</i>			
AD powder: thermally processed, dried, whole, ground <i>A. domesticus</i>			
Moisture	% w/w	76–82	≤ 5
Crude protein (N × 6.25)	% w/w	12–21	55–65
Fat	% w/w	3–12	29–35
Of which saturated	% w/w	36–45	36–45
Digestible carbohydrates	% w/w	0.1–2	1–4
Peroxide value	meq O ₂ /kg fat	≤ 5	≤ 5
Dietary fibre	% w/w	0.8–3	3–6
Chitin*	% w/w	≤ 3	≤ 10
Heavy metals			
Lead	mg/kg	≤ 0.05	≤ 0.05
Cadmium	mg/kg	≤ 0.06	≤ 0.06
Mycotoxins			
Aflatoxins (Sum of B1, B2, G1, G2)	µg/kg	≤ 0.3	≤ 0.3
Deoxynivalenol	µg/kg	≤ 20	≤ 20
Ochratoxin A	µg/kg	≤ 0.4	≤ 0.4
Sum of dioxins and dl-PCBs (UB,WHO-TEQ₂₀₀₅)	pg/g fat	≤ 1.25	≤ 1.25
Microbiological			
Total aerobic colony count	cfu/g	≤ 10 ⁵	≤ 10 ⁵
<i>Enterobacteriaceae</i> (presumptive)	cfu/g	≤ 100	≤ 100
<i>Escherichia coli</i>	cfu/g	≤ 50	≤ 50
<i>Listeria monocytogenes</i>	in 25 g	Not detected	Not detected
<i>Salmonella</i> spp.	in 25 g	Not detected	Not detected
<i>Bacillus cereus</i> (presumptive)	cfu/g	≤ 100	≤ 100
Coagulase positive -staphylococci	cfu/g	≤ 100	≤ 100
Sulfite-reducing anaerobes	cfu/g	≤ 30	≤ 30
Yeasts and Moulds	cfu/g	≤ 100	≤ 100

Cfu: colony forming units; UB: Upper Bound; Sum of dioxins and dl-PCBs (WHO-TEQ₂₀₀₅): sum of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls by using toxicity equivalence factors (TEF) set by World Health Organization in 2005.

*: 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) and Lao PDR (Codex Alimentarius Commission, 2010; Durst and Hanboonsong, 2015), but also in Cambodia (FAO, 2013a), Ghana (Anankware et al., 2016) and Mexico (Ramos-Elorduy, 2009).

Hanboonsong et al. (2013) reported that around 20,000 *A. domesticus* small- and medium-sized 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 tons 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).

domesticus is also farmed in Lao PDR (Hanboonsong and Durst, 2014), as well as to 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) (Swiss Federal Office of Food Safety, 2017). In Canada, it is considered non-novel for use as a food or food ingredient (Health Canada, 2021).

According to information provided by the applicant, *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.).

3.6.2. History of use of the NF

According to information provided by the applicant, the three NF formulations are already sold for human consumption in the Netherlands since 2018 and no adverse effects were reported.

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) are 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 level (g NF/100 g)	
			AD dried and AD powder	AD frozen
4	A005K	Bread and rolls with special ingredients added	10	30
3	A005Y	Crackers and breadsticks	10	30
3	A00EY	Cereal bars	15	30
4	A009X	Biscuits, sweet, plain	8	30
5	A007L	Dried pasta	1	3
5	A007Y	Dried stuffed pasta	15	30
4	A0CSK	Pre-mixes (dry) for baked products	15	30
5	A045N	Tartar sauce	10	30
5	A03VH	Potatoes and vegetables meal	5	15
5	A03VN	Hummus	5	15
5	A03VS	Beans and vegetables meal	5	15
5	A03ZV	Pizza and similar with cheese, and vegetables and fruits	5	15
5	A03ZT	Pizza and similar with cheese, and vegetables	5	15
5	A0CDP	Pasta, filled, cooked	5	15
4	A02PN	Whey powder	20	40
3	A03TE	Meat imitates	50	80
4	A0B9X	Tomato soup, dry	5	20

FoodEx2 level	FoodEx2 code	Food category	Max use level (g NF/100 g)	
			AD dried and AD powder	AD frozen
4	A0B9X	Mushroom soup, dry	5	15
4	A0B9R	Mixed vegetables soup, dry	5	15
4	A041P	Potato soup	5	15
4	A041M	Onion soup	5	15
4	A041Q	Legume (beans) soup	5	15
4	A041N	Tomato soup	5	15
4	A041R	Mushroom soup	5	15
5	A041S	Mixed vegetables soup	5	15
4	A042E	Caesar salad	5	15
4	A042H	Prepared pasta salad	5	15
5	A00FD	Tortilla chips	20	40
2	A03MA	Beer and beer-like beverage	1	1
2	A03PM	Mixed alcoholic drinks	1	1
2	A04QF	Unsweetened spirits and liqueurs	1	1
4	A0EQD	Chocolate and similar	10	30
3	A01BJ	Primary derivatives from nuts and similar seeds	25	40
5	A0BAV	Chickpeas (without pods)	25	40
3	A014C	Tree nuts	25	40
3	A015F	Oilseeds	25	40
3	A06HL	Snacks other than chips and similar	100	100
4	A02QC	Frozen yoghurt	5	15
5	A03XG	Meat balls	16	40
5	A03XF	Meat burger (no sandwich)	16	40

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). Since the applicant intends to use only one specific form of the NF (i.e. either AD frozen, AD dried or AD powder) in the respective food categories, the anticipated daily intake was calculated with the maximum use levels for AD dried/AD powder as this corresponds to the highest possible amount of dry matter in each food category. 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 estimate resulting from the use of the NF (AD dried/AD powder) 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 ^(b)	Highest ^(b)	Lowest ^(c)	Highest ^(c)
Infants	< 1	4	121	14	571
Young children ^(a)	1–< 3	47	416	182	1,033
Other children	3–< 10	39	249	157	759
Adolescents	10–< 18	21	130	83	515
Adults ^(d)	≥ 18	53	124	150	368

bw: body weight.

(a): Referred as toddlers in the EFSA food consumption comprehensive database (EFSA, 2011).

(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 23/3/2021. The lowest and the highest averages observed among all EU surveys are reported in these columns.

(c): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 23/3/2021. 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).

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

3.7.4. Estimate of exposure to undesirable substances

Based on the highest P95th 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 values for the concentration 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. The Panel considers that 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.

3.7.5. Possible physical hazards

The risk of intestinal constipation caused by large spines on the insect's tibia and chitinous material has been reported for *Locusta migratoria* (FAO, 2013a). As the spines in *A. domesticus* are smaller than in *L. migratoria*, the Panel notes that the risk for intestinal constipation is expected to be lower and can be further reduced by removing legs and wings for AD dried and for AD frozen or by reducing the particle size for AD powder.

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 formulations which consist mainly of protein, fat, dietary fibre (mainly chitin) and inorganic matter (AD dried, AD powder); water, protein, fat, dietary fibre (mainly chitin) and inorganic matter (AD frozen). The energy value of the NF is on average 482 and 2,240 kJ/100 g for AD frozen and AD dried, respectively (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 AD dried.

The NF contains on average 15.1 g and 60.3 g crude protein per 100 g AD frozen and AD dried, respectively, 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 *A. domesticus* due to the presence of considerable amounts of non-protein nitrogen derived mainly from chitin (Janssen et al., 2017). A study provided by the applicant identified a nitrogen-to-protein conversion factor of 5.57 for AD powder. Using this factor, the protein content of AD dried is about 11% 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 ISO 13903:2005 and Regulation (EC) No 152/2009 (Appendix A). The applicant also analysed the amount of amino acids in mg per g protein of AD dried. Results show that in the protein from AD dried, 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). For comparison, 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 AD dried was $67.3\% \pm 2.2\%$, compared to casein $75.3\% \pm 1.4\%$, indicating that proteins of *A. domesticus* are less bio-accessible than casein. Following the recommendation by FAO (2013b), the protein quality was determined as Digestible Indispensable Amino Acid Score (DIAAS). The DIAAS value for AD dried corresponded to 61%, compared to casein with a DIAAS of 91%. Sulfur amino acids (methionine + cysteine) were the limiting amino acids.

If the NF entirely replaces other protein sources of higher quality, it may negatively impact on protein nutrition if the overall protein intake is low. Based on the high (95th percentile) intake levels of the NF (Section 3.7.3, Table 9) with a maximum content of protein of 65% (AD dried) (Section 3.5, Table 7), the corresponding protein intake per kg bw per day from the NF would amount to 0.37 g for infants, 0.67 g for young children, 0.49 g for other children, 0.33 g for adolescents and 0.24 g for adults. These intakes would correspond to up to 28%, 59–74%, 57–54%, 37–40% and 29% of respective dietary reference values (DRVs) (EFSA NDA Panel, 2012) for protein for infants, young children, other children, adolescents and adults, respectively. 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 consumption of the NF should not negatively impact protein nutrition.

The major fatty acids in the AD dried are palmitic acid, linoleic acid and oleic acid (Appendix B). On average saturated, monounsaturated fats and polyunsaturated fatty acids constitute 40.8%, 28.9% and 30.3% of total fatty acids, respectively. The average content of trans fatty acid is 0.6% of total fatty acids.

The applicant provided analytical data on the levels of some minerals and vitamins, and after EFSA's request, on boron, molybdenum, iodine and selenium (Table 10).

Table 10: Content of micronutrients (minerals and vitamins) in the NF (AD dried)

Parameter	Analytical method	Batch number					
		#6	#7	#8	#9	#10	
Minerals (units)							
Sodium (mg/100 g)	ICP-MS	280	280	280	280	280	
Calcium (mg/100 g)		150	140	140	140	140	
Phosphorous (mg/100 g)		710	710	710	690	710	
Magnesium (mg/100 g)		64	63	64	63	64	
Potassium (mg/100 g)		640	650	640	650	660	
Iron (mg/100 g)		6.8	6.6	6.7	6.9	6.7	
Manganese (mg/100 g)		3.9	3.9	3.8	3.8	3.9	
Copper (mg/100 g)		2.9	2.8	2.9	2.9	2.9	
Zinc (mg/100 g)		24	24	24	24	23	
Iodine ($\mu\text{g}/100\text{ g}$)		57	52	46	42	40	
Selenium ($\mu\text{g}/100\text{ g}$)		43	43	44	< 25	< 25	
Boron (mg/100 g)		ICP-OES	0.13	0.17	0.15	0.15	0.3
Molybdenum (mg/100 g)			< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Vitamins (units)							
Retinol ($\mu\text{g}/100\text{ g}$)	EN 12823-1 2014	< 21	< 21	< 21	< 21	< 21	
Thiamin (mg/100 g)	EN 14122:2003	0.25	0.24	0.24	0.24	0.24	
Thiamin HCL (mg/100 g)	EN 14122:2003	0.32	0.30	0.31	0.31	0.31	
Vitamin B12 ($\mu\text{g}/100\text{ g}$)	AOAC 2008.91:4	4.18	4.37	3.76	4.31	4.01	
Riboflavin (mg/100 g)	EN 14152:2003	1.05	0.93	1.01	0.99	0.95	

Parameter	Analytical method	Batch number				
		#6	#7	#8	#9	#10
Niacin (mg/100 g)	EN 15652:2009	3.98	4.44	4.51	4.37	3.98
Pantothenic acid (mg/100 g)	AOAC 2012.16	4.37	4.31	4.30	4.42	4.35
Pyridoxine hydrochloride ($\mu\text{g}/100\text{ g}$)	EN 14164	96	99	94	96	103
Biotin ($\mu\text{g}/100\text{ g}$)	LST AB 266.1, 1995	89.8	105	97.3	102	112
Folate ($\mu\text{g}/100\text{ g}$)	NMKL 111:1985	167	149	158	161	163
Cholecalciferol ($\mu\text{g}/100\text{ g}$)	EN 12821:2009	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25
Alpha-tocopherol (mg/100 g)	EN 12822:2014	4.26	4.28	4.34	3.94	3.68

LOQ: limit of quantification; ICP-MS: Inductively coupled plasma mass spectrometry; ICP-OES: Inductively coupled plasma-atomic emission spectroscopy.

Considering the mean contents reported in Table 10 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 group.

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 7.2 g chitin in 100 g AD dried formulation (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 and hydrogen cyanide (Meyer-Rochow et al., 2021; 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 AD dried (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: Levels of antinutrients in the NF (AD dried)

Parameter (unit)	Analytical method	#6	#7	#8	#9	#10
Total polyphenols (%)	Folin-Ciocalteu	0.75	0.76	0.81	0.76	0.72
Tannins (%)	Folis Denis	0.7	0.7	0.7	0.7	0.7
Oxalic acid (mg/kg)	HPLC/UV, in house method	< 100	< 100	< 100	< 100	< 100
Phytic acid (g/kg)	ANAL-10445	1.3	1.4	1.0	1.3	1.1
Hydrogen cyanide (mg/kg)	NEN-EN 16160	< 5	< 5	< 5	< 5	< 5
Trypsin inhibitor (mg/g)	NEN-EN-ISO 14902	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5

HPLC: high-performance liquid chromatography.

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

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

Regarding the safety of chitin present in NF, the applicant referred to EFSA's scientific opinion on the safety of 'chitin-glucan' as an 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*.

As reviewed by Komi et al. (2018), chitin has been reported 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. 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).³ No firm conclusions can be drawn by the Panel since only the abstract was accessible (EFSA NDA Panel, 2021).

The applicant performed a literature search and referred to one paper retrieved from the literature reporting one acute and subchronic toxicity study conducted with *Gryllus bimaculatus* in compliance with GLP practices, OECD guidelines and Korean guidelines (Ryu et al., 2016). The Panel notes that *G. bimaculatus* belongs to the same family as *A. domesticus* (Gryllidae), but these are different species and also the rearing conditions of the insects used in the study are not known. Therefore, even though the study did not show adverse effects, it cannot be considered for the toxicological assessment of the NF.

3.10.1. Genotoxicity

The applicant reported that it was not possible to perform an *in vitro* micronucleus test and a bacterial reverse mutation test, due to the lack of solubility of the NF (AD dried) in water, DMSO, ethanol and acetone at the lowest concentration tested of 10 mg/mL.

3.10.2. Cytotoxicity

A cell toxicity assay was performed testing AD 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

The applicant did not provide any subacute or subchronic studies conducted with the NF.

3.10.4. Human data

The applicant did not provide any human studies conducted with the NF.

3.11. Allergenicity

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

A. domesticus belongs to Hexapoda (Insecta class), one of the four subphyla of Arthropoda. 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 allergens 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% of all anaphylactic food-related reactions reported in China between 1980 and 2007. Furthermore, the occurrence of anaphylactic reactions due to consumption of fried insects (grasshoppers and crickets) has been reported in Thailand (Piomrat et al., 2008).

By using a proteomic and bioinformatic approach, Barre et al. (2021) identified 46 proteins in *A. domesticus*, corresponding to pan-allergens which develop cross-reactivity with other homologous

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

proteins present in arthropods, but also a few other allergens less abundant or even lacking in arthropoda, such as the odorant-binding protein. Tropomyosin appeared as a major pan-allergen largely distributed among dust mites, insects, crustaceans and molluscs, as also confirmed by Sokol et al. (2017). De Marchi et al. (2021) reported that tropomyosin from *A. domesticus* was more stable to *in vitro* gastric digestion than the shrimp homologue.

Cross-reactivity to *A. domesticus* 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; De Marchi et al., 2021). 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; Broekman et al., 2017; Rougé and Barre, 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) reviewed 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 *A. domesticus*, as it might include common food allergens. 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 *A. domesticus* despite implementing a fasting step.

A frequently reported case of allergic symptoms to insects, including *A. domesticus*, relates to occupational exposure (Bagenstose et al., 1980; Linares et al., 2008; Park et al., 2014).

The Panel considers that the consumption of the NF might trigger primary sensitisation to *A. domesticus* proteins. The Panel also considers that allergic reactions may occur in subjects allergic to crustaceans, mites and molluscs (cross-reactivity). Furthermore, the 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 house cricket (*A. domesticus*), frozen, dried or 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 composition of AD dried/AD powder differs from to the one of AD frozen due to the reduced water content in the dried formulations (AD dried/powder are concentrates of AD frozen). The NF consists mainly of protein, fat, dietary fibre (mainly chitin) and inorganic matter (AD dried and AD powder); water, protein, fat, dietary fibre (mainly chitin) and inorganic matter (AD 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 182 to 1,033 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 (AD dried) is $67.3\% \pm 2.2\%$, with a DIAAS value of 61%. The limiting amino acids were the sulfur-containing ones. 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 for the analysed micronutrients are expected to be 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. 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 with the NF were provided by the applicant. Considering that no safety concerns arise from the history of use of *A. domesticus* or from the compositional data of the 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. 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 detailed description of the production process, proximate analysis, contaminants analysis, stability studies, microbiological analysis, data on Novel Food sales, solubility of test item for the genotoxicity tests, digestibility of protein study report and the cytotoxicity/cellular toxicity study report claimed as 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 22 August 2019 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of whole and ground crickets (*Acheta domesticus*) as a novel food. Ref. Ares (2019)5356758 22/08/2019.
- 2) On 22 August 2019, a valid application on whole and ground crickets (*Acheta domesticus*), 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 2018/0804) and the scientific evaluation procedure was initiated.
- 3) On 11 October 2019, 12 March 2020, 18 January 2021, 22 April 2021 and 16 June 2021 EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
- 4) On 17 January 2020, 12 October 2020, 6 April 2021, 7 May 2021 and 18 June 2021, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
- 5) On 02 June 2021, EFSA received a letter from the European Commission with the revised request for a scientific opinion on frozen and dried formulations from whole house crickets (*Acheta domesticus*) as a novel food. Ref. Ares (2021)4080152.

- 6) During its meeting on 7 July 2021, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of frozen and dried formulations from whole house crickets (*Acheta domesticus*) as a NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

ACFS	Thailand's National Bureau of Agricultural Commodity and Food Standards
AMCase	acidic mammalian chitinase
AD	<i>Acheta domesticus</i>
AddV	cricket densovirus
ADF	acid detergent fibre
ADLv	acid detergent lignin
ADME	absorption, distribution, metabolism and excretion
ANF	antinutritional factors
AOAC	Association of Official Analytical Chemists
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
CrPV	cricket paralysis virus
DIAAS	digestible indispensable amino acid score
DMSO	dimethyl sulfoxide
DRVs	dietary reference values
FAO	Food and Agriculture Organization
FFA	free fatty acids
FSANZ	Food Standards Agency Australia New Zealand
GC-MS/MS	gas chromatography-tandem mass spectrometry
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practice
HACCP	hazard analysis critical control points
HPLC	high performance liquid chromatography
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
IL	interleukin
ISO	International Organization for Standardization
LC-MS/MS	liquid chromatography/tandem mass spectrometry
Lao PDR	Lao People's Democratic Republic
LOQ	limits of quantification
MCPD	monochloropropane diol
MUFA	mono-unsaturated fatty acids
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
N	nitrogen

ND	not detected
NF	novel food
NVWA	Netherlands Food and Consumer Product Safety Authority
OECD	Organization for Economic Co-operation and Development
PA	p-anisidine value
PAH	polycyclic aromatic hydrocarbons
PCBs	polychlorinated biphenyls
Pmerg DNV	<i>Penaeus merguensis</i> densovirus
PUFA	poly-unsaturated fatty acids
PV	peroxide value
Sum of dioxins and dl-PCBs (WHO-TEQ ₂₀₀₅)	sum of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls by using toxicity equivalence factors (TEF) World Health Organization in 2005
UB	upper bound
UV	ultraviolet
WHO	World Health Organisation
w/w	weight per weight

Appendix A – Batch-to-batch amino acid analysis of AD dried

Amino acids (mg/100 g protein)	#6	#7	#8	#9	#10	FAO (2013b)
Essential						
Histidine	24.9	25.3	25.2	24.5	25.3	15
Isoleucine	41.6	41.2	42.3	42.1	42.0	30
Leucine	78.0	78.9	78.0	77.3	77.4	59
Lysine	62.5	65.1	64.2	62.9	63.7	45
Methionine	19.7	18.9	18.3	18.7	18.8	22*
Phenylalanine	39.4	39.8	40.2	39.2	39.6	38**
Threonine	42.7	42.9	43.1	42.8	42.9	23
Tryptophan	10.7	10.8	10.8	11.0	11.4	6
Valine	64.4	63.9	64.3	63.1	64.7	39
Conditionally essential						
Arginine	67.2	68.1	68.0	66.4	66.6	
Cystein + cystine	9.6	9.6	9.3	10.0	9.8	
Glycine	55.9	55.5	55.4	54.8	55.4	
Proline	62.7	59.4	59.7	73.6	62.2	
Tyrosine	72.1	72.1	71.2	69.9	70.9	
Non-essential						
Alanine	93.3	91.5	91.2	91.1	91.4	
Aspartic acid	90.4	90.8	90.9	88.1	90.7	
Glutamic acid	119.8	121.0	122.8	119.1	122.0	
Serine	45.1	45.2	45.2	45.4	45.2	

All amino acids were analysed according to method ISO 13903:2005 with the exception of tryptophan analysed according to method EU 152/2009.

*: Methionine + cysteine.

** : Phenylalanine + tyrosine.

Appendix B – Batch-to-batch fatty acid analysis of AD dried

Fatty acids (% of total fatty acids)	#6	#7	#8	#9	#10
Myristic acid	0.9	0.9	0.9	0.9	0.8
Palmitic acid	30.6	30.6	30.5	30.6	30.7
Stearic acid	8.5	8.4	8.4	8.4	8.3
Oleic acid	26.1	26.2	26.3	26.3	26.4
Linoleic acid	26.6	26.7	26.6	26.6	26.7
Alpha linolenic acid	3.0	3.0	3.0	3.0	3.0
Saturated fatty acid	41.1	40.8	40.8	40.8	40.8
MUFA	28.6	28.7	28.7	28.7	28.9
PUFA	30.4	30.5	30.4	30.5	30.3
Trans Fatty Acid	0.6	0.6	0.6	0.6	0.6
Omega 3	3.3	3.4	3.3	3.4	3.4
Omega 6	26.8	26.9	26.8	26.8	26.9

MUFA: mono-unsaturated fatty acids; PUFA: poly-unsaturated fatty acids.

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.online.library.wiley.com/doi/10.2903/j.efsa.2021.6779#support-information-section>).