Safety of frozen and freeze-dried formulations of the lesser mealworm (Alphitobius diaperinus larva) as a Novel food pursuant to Regulation (EU) 2015/2283

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Safety of frozen and freeze-dried formulations of the lesser mealworm (*Alphitobius diaperinus* larva) 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 frozen and dried formulations from whole lesser mealworm (*Alphitobius diaperinus* larva) as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The term lesser mealworm refers to the larval form of the insect species *Alphitobius diaperinus*. The NF comprises the frozen and freeze-dried formulations of the lesser mealworm as whole or in the form of a paste or powder. Apart from water in the frozen formulations (whole, paste), the main components of the NF are crude protein and fat, besides smaller amounts of digestible carbohydrates and fibre (chitin). The Panel notes that the levels of contaminants in the NF depend on the concentration of such substances in the insect feed. The Panel notes furthermore that 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 formulations added as an ingredient to various food products such as cereal bars, pasta, meat imitates and bakery products. The target population is the general population. Additionally, the applicant proposed to use the NF as a food supplement in adults. The Panel notes that, considering that the NF will not be the sole source of dietary protein, and the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous. The submitted subchronic 90-day toxicity study with the NF as testing material did not raise safety concerns. The Panel considers that the consumption of the NF may induce primary sensitisation and allergic reactions to lesser mealworm proteins and may cause allergic reactions in subjects with allergy to crustaceans and dust mites. Additionally, allergens from the feed may end up in the NF. Allergenicity aside, the Panel concludes that the NF is safe under the proposed uses and use levels.

Keywords: Novel foods, food safety, *Alphitobius diaperinus* larva, lesser mealworm, insect powder, entomophagy, food supplement

Requestor: European Commission

Question number: EFSA-Q-2018-00282

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1. Introduction

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

On 7 January 2018, the company Proti-Farm Holding NV[1] submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) No 2015/2283' to place whole and ground lesser mealworm (*Alphitobius diaperinus*) larvae products on the Union market as a novel food.

In the process of the evaluation of this novel food, it became apparent that the Commission should amend the title of the mandate in relation to the terms "mealworm", "larvae", "grinded" and "products". The term "mealworm" already indicates the developmental stage of the insect, i.e., larva. Therefore, it is appropriate to correct the sentence by including the term "larva" together with the scientific (*Alphitobius diaperinus*). The term "grinded" should be replaced by "ground" and the term "products" should also be removed as it does not clearly inform of the nature of the novel food. On that basis, it is appropriate to amend the title of the request.

The novel food (frozen and freeze-dried formulations of the lesser mealworm as whole, in the form of a paste, or powder) is intended for use in various foodstuffs such as breakfast cereals and bars, pasta and noodles, bread and rolls, fine bakery wares, processed meat, soups and broths, snacks, meat imitates, and chocolate/cocoa-based products. The target population is the general population. In addition, it is also intended for use in food supplements for the adult population.

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 frozen and freeze-dried formulations of the lesser mealworm (*Alphitobius diaperinus* larva) as whole, in the form of a paste, or powder as a novel food.

In addition, the European Food Safety Authority is requested to include in its scientific opinion a statement as to if, and 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.

On that basis, the Commission amended the title to "Revised request for a scientific opinion on frozen and freeze-dried formulations of the lesser mealworm (*Alphitobius diaperinus* larva) as a novel food" and amended the part describing the proposed uses.

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. During the assessment, the Panel identified additional data which were not included in the application.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469.[2]

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

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data requested by the applicant to be protected comprise compositional data, stability studies, an *in vitro* protein digestibility study and a subchronic 90-day toxicity study.

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

[1] During the assessment, the applicant's company name changed from 'Proti-Farm Holding NV' to 'Ynsect NL B.V.'.

[2] Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 64–71.

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 Commission Implementing Regulation (EU) 2017/2469.

Additional information that 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).

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 that is the subject of the application is frozen and freeze-dried forms of the *Alphitobius diaperinus* larva (lesser mealworm), an insect species that belongs to the family of Tenebrionidae (darkling beetles). The NF falls under the category 'food consisting of, isolated from or produced from animals or their parts' as described in Article 3(2)(v) of Regulation (EU) 2015/2283. The NF is produced by farming and processing lesser mealworms. The frozen forms consist mainly of water, crude protein and fat, whereas the dried forms consist mainly of crude protein, fat, digestible carbohydrates and dietary fibre. The NF is proposed to be used as whole frozen or whole dried insect, as a paste, or in the form of powder, added as an ingredient to various food products such as cereal bars, pasta, soups and bakery products. The NF will be added to foods intended for the general population. Additionally, the NF is intended to be added as an ingredient in food supplements for the adult population.

3.2. Identity of the NF

The NF comprises frozen and freeze-dried forms of whole or ground lesser mealworm. The term 'lesser mealworm' refers to the larval form of *Alphitobius diaperinus* (Panzer, 1797), an insect species that belongs to the family of Tenebrionidae (darkling beetles). The following scientific synonyms have been described (Aalbu et al., 2002; Dunford and Kaufman, 2006): *Alphitobius diaperinus* (Panzer), Wollaston (1854); Alphitobius mauritanicus (Curtis), Stephens (1832); *Cryptops Solier* (1851); *diaperinus* (Panzer) (1797); *Heterophaga diaperina* (Panzer), *Redtenbacher* (1849); *Heterophaga opatroides* (Dejean), Dejean (1833); Heterophaga Redtenbacher (1845); *Phaleria diaperinus* (Panzer), Latreille (1804); *Proselytus caffer* Fahraeus 1870; *Tenebrio diaperinus* Panzer (1797); *Uloma mauritanica* Curtis (1831); *Uloma opatroides Dejean* (1821). Apart from 'lesser mealworm', other common names identified are 'buffalo worm', 'litter beetle' and 'ténébrion (petit) mat'.

A. diaperinus sp. is currently present worldwide, with sub-Saharan Africa (Geden and Hogsette, 1994) and the tropical east African region (Lambkin, 2001) being its two hypothesised points of origin. According to Dunford and Kaufman (2006), *A. diaperinus* sp. was introduced to North America via Europe. This insect species is generally considered as a pest, mainly of grain products (Spilman, 1991; Hosen et al., 2004). Furthermore, both larvae and adults occur frequently in poultry farms and henneries, since humid and warm conditions favour their growth (Francisco and do Prado, 2001; Lambkin, 2001).

The NF is intended to be marketed as (a) whole blanched and frozen *A. diaperinus* larva (ADL frozen); (b) paste from whole blanched, ground and frozen *A. diaperinus* larva (ADL paste); (c) whole blanched and freeze-dried *A. diaperinus* larva (ADL dried); and (d) powder from whole blanched, freeze-dried and ground *A. diaperinus* larva (ADL powder). The entire mealworms are meant for human consumption, no parts are removed. 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, in accordance with ISO 22000 requirements. The production process can be divided into three distinctive parts, i.e. farming, harvesting and post-harvest processing.

The initial livestock of *A. diaperinus* was obtained from a breeding facility where it has been bred for about 40 years. Farming includes mating of the adult insect population and rearing of the larvae. The eggs are separated from the adult insects and are hatched separately. After being hatched from the eggs, the brown/light-brown larvae grow in dedicated containers made of certified food contact

hard-type polypropylene and polyethylene. This reduces the probability of plastic ingestion by the larvae (EFSA NDA Panel, 2021a,b). The applicant stated that no veterinary medicinal products are used during the rearing of the larvae.

Larvae of the Tenebrionidae family can bioaccumulate chemical agents such as heavy metals, pesticide residues and other undesirable compounds [e.g. polychlorinated biphenyls (PCBs), dioxins] through their feed intake (Bednarska and Świątek, 2016; Ghannem et al., 2018; Houbraken et al., 2016; Lindqvist and Block, 1995; Van der Fels-Klerx et al., 2016; Vijver et al., 2003). The applicant reported that the feed administered to the insects is of plant origin, compliant with the provisions of Regulation (EU) No 68/2013 on the Catalogue of feed materials and produced according to good manufacturing practices (GMP). Moreover, the applicant informed that the feed substrate used may contain gluten-containing grains.

A. diaperinus can be infected by or be a vector for zoonotic agents such as parasites, entomopathogenic fungi, bacteria and viruses (Rumbos et al., 2018; Smith et al., 2021). For example, this insect species may be infected with the protozoan *Histomonas meleagridis* (Huber et al., 2007), with the nematodes *Subulura brumpti* (Karunamoorthy et al., 1994) and *Hadjelia truncate* (Alborzi and Rahbar, 2012), and the tapeworm *Choanotaenia infundibulum* (Elowni and Elbihari, 1979). *A. diaperinus* can harbour viruses that infect honeybees such as the Israeli acute paralysis virus (IAPV) and the black queen cell virus (BQCV), and several avian viruses (Bertola and Mutinelli, 2021) such as the reovirus (De las Casas et al., 1972). However, these viruses are specific to insects, and are not pathogenic for humans or other vertebrates (EFSA Scientific Committee, 2015). The applicant stated that measures to control the contamination of the rearing facilities by pests and rodents, which could enhance the presence of zoonotic agents, are in place.

During the rearing of the larvae, deceased insects and faecal contamination are monitored and removed. Deceased larvae, which have a darker colour compared to live larvae, are removed after visual inspection. A 24-h fasting step is implemented, to allow the larvae to discard their bowel content. Mechanical sieving is used to harvest the larvae, separating them from the substrate, exuvia and faeces.

The post-harvesting processing includes the rinsing of the larvae with water, killing of the larvae by blanching (immersion in boiling water), cooling down of the larvae and removal of the excess water. The thermal treatment contributes to the reduction of the microbial load of the larvae as well as to the elimination of potentially present viruses and parasites. Furthermore, this step reduces the activity of enzymes (e.g. tyrosinase/phenoloxidase) (Janssen et al., 2017a) which may induce enzymatic browning in the larvae of the Tenebrionidae family (Nappi and Vass, 1993; Nappi and Ottaviani, 2000; Sugumaran et al., 2000; Nappi and Christensen, 2005).

Freezing of the blanched larvae, packing and storage at −18°C result in 'ADL frozen'. Grinding (below 0°C) of the blanched larvae, freezing of the resulted paste, packing and storage at −18°C result in 'ADL paste'.

ADL frozen can also undergo freeze-drying (final moisture content ≤ 5%) and is either stored at 5°C as 'ADL dried' or undergoes a grinding step and is stored at 5°C in the form of powder (ALD powder).

The Panel considers that the production process is sufficiently described.

3.4. Compositional data

In order to confirm that the manufacturing process is reproducible and adequate to produce on a commercial scale a product with certain characteristics, the applicant provided qualitative and quantitative data on chemical and microbiological parameters for a number of different batches of the NF forms i.e. (a) ADL frozen; (b) ADL paste; (c) ADL dried; (d) ADL powder.

For all parameters, at least five independently produced batches were analysed. Considering the production process, the Panel considers that the forms of the NF, ADL dried and ADL powder are representative of each other regarding most of their compositional parameters, excluding microbiological aspects and oxidative status of fats. The same consideration has been made for ADL frozen and ADL paste. Grinding increases the surface area of the NF and makes ADL powder and ADL paste more prone to microbiological growth and deterioration, compared to ADL dried and ADL frozen, respectively.

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 have 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, 2016).

ADL frozen and ADL paste consist mainly of water, crude protein, fat and small amounts of digestible carbohydrates and fibre (chitin). In the dried formulations (ADL dried/ADL powder), the concentrations of all components, excluded water, are higher, compared to ADL frozen/ADL paste due to the reduced water content. The results of the proximate analyses of the NF are presented in Tables 1 and 2. The amino acid, fatty acid, vitamin and mineral composition are reported in Section '3.9 Nutritional information'.

Table 1: Batch-to-batch analysis of the undried NF forms

Parameter (unit)	Batch number (ADL frozen)					Batch number (ADL paste)					Analytical method
	#70	#71	#80	#81	#82	#17	#18	#40	#42	#44	
Crude protein (g/100 g of NF)	20.3	18.6	20.0	19.4	18.4	17.88	16.78	17.86	19.71	16.54	Kjeldahl, GAFTA method 4:1-M[(b)]; ISO 5983-M[(c)]
Fat (g/100 g of NF)	9.53	8.21	7.25	8.15	9.22	6.68	6.87	6.39	5.57	5.42	Soxhlet, GAFTA method 3:0-M
Total digestible carbohydrates (g/100 g of NF)	1.01	1.06	0.55	0.83	0.67	/	/	/	/	/	Luff Schoorl
Dietary fibre[(a)] (g/100 g of NF)	1.60	1.5	1.3	1.5	1.2	2.26	2.01	2.18	2.67	1.97	ADF, GAFTA Method 9:0-M[(d)]
Ash (g/100 g of NF)	1.10	1.06	1.11	1.12	1.17	0.9	0.90	0.92	1.02	0.87	ISO 5984-M; GAFTA Method 11:0-M
Moisture (g/100 g of NF)	66.8	70.1	70.3	69.5	69.8	71.6	71.8	72.1	71.3	74.7	ISO 6496-M; GAFTA method 2:1-M
Energy (kcal/100 g of NF)	170	160	150	160	160	/	/	/	/	/	Regulation (EU) 1169/2011
Energy (kJ/100 g of NF)	710	670	630	670	670	/	/	/	/	/	Regulation (EU) 1169/2011

(a): Chitin is the main form of dietary fibre in the NF.
(b): GAFTA: Grain and Feed Trade Association.
(c): ISO: International Organization for Standardization.
(d): ADF: Acid Detergent Fibre.
/: not provided.

Table 2: Batch-to-batch analysis of the dried NF forms

Parameter (unit)	Batch number (ADL dried)				Batch number (ADL powder)									Analytical method
	#43	#46	#47	#51	#39	#48	#49	#50	#69	#77	#78	#79	#118	
Crude protein (g/100 g of NF)	58.76	60.49	61.82	62.67	61.95	59.87	63.15	61.94	61.5	60.6	61	60.7	59.1	Kjeldahl, GAFTA method 4:1-M[(b)]; ISO 5983-M[(c)]
Fat (g/100 g of NF)	25.9	26.6	25.7	23.5	25.2	26.2	22.9	22.9	23.5	26.8	26.2	24.9	27.3	Soxhlet, GAFTA method 3:0-M
Total digestible carbohydrates (g/100 g of NF)	/	/	/	/	/	/	/	/	/	2.38	2.18	2.82	3.21	Luff Schoorl
Dietary fibre[(a)] (g/100 g of NF)	6.08	6.47	6.63	6.97	6.57	6.48	7.25	7.29	6.72	4.1	4.1	4.2	4.3	ADF, GAFTA Method 9:0-M[(d)]

Parameter (unit)	Batch number (ADL dried)				Batch number (ADL powder)									Analytical method
	#43	#46	#47	#51	#39	#48	#49	#50	#69	#77	#78	#79	#118	
Ash (g/100 g of NF)	3.5	3.58	3.72	3.56	3.64	3.44	3.61	3.54	3.40	3.60	3.59	3.54	3.39	ISO 5984-M; GAFTA Method 11:0-M
Moisture (g/100 g of NF)	2.74	1.98	<0.3	2.38	1.08	2.43	2.7	3.44	4.14	3.72	3.90	4.20	4.17	ISO 6496-M; GAFTA method 2:1-M
Energy (kcal)	/	/	/	/	/	/	/	/	/	500	500	490	500	Regulation (EU) 1169/2011

(a): Chitin is the main form of dietary fibre in the NF.
(b): GAFTA: Grain and Feed Trade Association.
(c): ISO: International Organization for Standardization.
(d): ADF: Acid Detergent Fibre.
/: not provided.

Given the possible variations in rearing conditions [feed, developmental stage at the time of harvesting, ambient conditions (Oonincx et al., 2015; Rumpold and Schlüter, 2013)] and the use of whole insects, the variation of compositional values is acceptable.

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

Chitin is the main form of carbohydrates in *A. diaperinus* larvae (Kurečka et al., 2021). It is a linear polysaccharide constituted by β-(1,4)-linked 2-amino-2-deoxy-β-D-glucopyranose and 2-acetamido-2-deoxy-β-D-glucopyranose units (Muzzarelli, 1973; Roberts, 1992). The applicant provided analytical data on the levels of chitin in five independently produced batches of ADL paste and five of ADL dried. 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 measured as acid detergent fibre (ADF). According to Finke (2007), ADF values are an overestimation of insects' chitin content since the ADF fraction contains a significant amount of amino acids, meaning that chitin content is possibly lower than the reported amounts. Mean ADF values were reported to be 2.2 (± 0.19) g/100 g for ADL paste and 7.84 (± 0.62) g/100 g for ADL dried (Table 3).

Table 3: Chitin content in the NF (ADL paste, ADL dried)

	Batch Number (ADL paste)					Batch Number (ADL dried)					Analytical method
	#55	#63	#64	#65	#67	#22	#23	#24	#25	#26	
Chitin content[(a)] (g/100 g NF)	2.1	2.1	2.4	2.0	2.4	8.9	7.8	7.3	7.7	7.5	Gravimetry ANAL-10351

(a): Calculated as ADF.

Concentrations of heavy metals in ADL paste, ADL dried and ADL powder analysed by ICP-MS are reported in Table 4. The applicant compared the values to the maximum levels for other foods as set in Regulation (EC) No 1881/2006[3]. The Panel notes that the levels of heavy metals reported for the NF are comparable to those set for other foods, as well as to those previously reported and assessed for other foods derived from whole insects (EFSA NDA Panel, 2021a,b,c,d), and that in the current EU legislation, no maximum levels of heavy metals are set for insects and products thereof as food.

[3] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5–24.

Table 4: Heavy metals in the NF (ADL paste, ADL dried, ADL powder)

Heavy metals (mg/kg)	Batch number (ADL paste)					Analytical method
	#63	#64	#65	#66	#67	
Arsenic	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	ICP-MS[(a)]
Cadmium	0.006	0.008	0.006	< 0.005	< 0.005	
Mercury	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	
Lead	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	
	Batch number (ADL dried)					
	#7	#8	#9	#88	/	
Arsenic	0.03	0.03	0.03	0.04	/	
Cadmium	0.014	0.013	0.019	0.026	/	
Mercury	0.0008	0.0007	0.0008	0.0011	/	
Lead	< 0.01	< 0.01	< 0.01	< 0.02	/	
	Batch number (ADL powder)					
	#6	#58	#59	#61	#62	
Arsenic	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	
Cadmium	0.019	0.026	0.022	0.02	0.021	
Mercury	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
Lead	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	

/: not provided.
(a): ICP-MS: Inductively coupled plasma mass spectrometry.

Analytical data on the levels of aflatoxins B1, B2, G1, G2, ochratoxin A, nivalenol, deoxynivalenol and zearalenone in ADL dried were provided (Table 5). Values were lower than the maximum levels set for different foods in Regulation (EC) No 1881/2006. The Panel notes that in the current EU legislation, no maximum levels of mycotoxins are set for insects as food.

Table 5: Mycotoxins in the NF (ADL dried)

Mycotoxins (μg/kg)	Analytical method	Batch number (ADL dried)				
		#10	#11	#12	#13	#14
Aflatoxin B1	IAC-LC/FLD[(a)]	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10
Aflatoxin B2		< 0.04	< 0.04	< 0.04	< 0.04	< 0.04
Aflatoxin G1		< 0.10	< 0.10	< 0.10	< 0.10	< 0.10
Aflatoxin G2		< 0.06	< 0.06	< 0.06	< 0.06	< 0.06
Aflatoxin (Sum of B1, B2, G1, G2)		< 0.30	< 0.30	< 0.30	< 0.30	< 0.30
Deoxynivalenol	IAC-LC/UV[(b)], EN 15891	< 40	< 40	< 40	< 40	< 40
Zearalenone	IAC-HPLC/UV[(c)], NEN-EN 15850-M	< 10	< 10	< 10	< 10	< 10
Ochratoxin A	IAC-LC-FLD	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4

(a): IAC-LC-FLD: Liquid Chromatography with fluorescence detector, with immunoaffinity column.
(b): IAC-LC/UV: Liquid Chromatography with ultraviolet detector, with immunoaffinity column.
(c): IAC-HPLC/UV: High Performance Liquid Chromatography with ultraviolet detector, with immunoaffinity column.

Regarding the occurrence of organic contaminants (e.g. dioxins and dioxin-like compounds, flame retardants, PCBs), the applicant did not provide analytical data in the NF but, instead, referred to the data from Poma et al. (2017) on samples of undried whole lesser mealworm. Additionally, the applicant stated that the levels of chemical contaminants are regularly monitored in the feed which is GMP$^+$ certified. The Panel notes that in the current EU legislation, no maximum levels of dioxins and dioxin-like compounds are set for insects as food.

Analytical data on the pesticide levels of five independently produced batches of ADL dried have been provided. The results showed that all the tested pesticides in the NF were below the limits of detection (LOD) of both analytical methods implemented (GC-MS/MS, LC-MS/MS).

Given the vegetable origin of the feeding 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 histamine for five independently produced batches of ADL powder (histamine quantification by ELISA). The range of reported values was 14–19 mg/kg (time 0) and < 2.9–2.5 mg/kg (time 30 days, accelerated conditions) (Table 11). The histamine values are much lower compared to the limit of 200 mg/kg for histamine in fishery products set in Regulation (EC) No 2073/2005. 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).

The applicant provided microbiological data on several independently produced batches of the NF produced at different time points between 2014 and 2017 that showed high variability in the total colony count. In order to address EFSA's request linked to this high variation, the applicant provided additional microbiological data on all NF forms (ADL frozen, ADL paste, ADL dried, ADL powder) from several independently produced batches produced at different time points between 2018 and 2020 (Table 6). The Panel observed that there was considerably less variability in the microbiological values of the new NF batches, compared to those initially analysed and also that the values did not exceed the specification limits.

Table 6: Microbiological analyses of the NF

Parameter (unit)	Analytical method	Batch number (ADL frozen)					Batch number (ADL paste)				
		#21	#32	#112	#116	#117	#1	#2	#3	#4	#5
Total colony count (cfu/g)	NEN-EN-ISO 4833 (ML/AL/42)	7,300	1,400	3,000	4,300	3,900	3,250	3,250	8,000	940	7,000
***Bacillus cereus* (cfu/g)**	ISO 7932 NEN 6875 (ML/AL/21)	< 10	10	< 10	10	< 10	< 10	< 10	< 10	< 10	< 10
Enterobacteriaceae (cfu/g)	NEN-ISO 21528-2 (ML/AL/55)	< 10	< 10	< 10	30	40	< 10	< 10	< 10	< 10	< 10
***Escherichia coli* (cfu/g)**	NEN-ISO 16649-2 (ML/AL/41)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
***Listeria monocytogenes* in 25 g (cfu/g)**	NEN-EN-ISO 11290-1 (ML/AL/15)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
***Salmonella* spp. in 25 g (cfu/g)**	NEN-EN-ISO 6579 (ML/AL/61)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
***Staphylococcus aureus* (cfu/g)**	NEN-EN-ISO 6888-1 (ML/AL/60)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
***Clostridium perfringens* (cfu/g)**	ISO 7937 (ML/AL/35)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Yeasts (cfu/g)	NEN-ISO 21527-1 (ML/AL/62), ISO 7954:1987	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Moulds (cfu/g)	NEN-ISO 21527-1 (ML/AL/62), ISO 7954:1987	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10

Parameter (unit)	Analytical method	Batch number (ADL dried)					Batch number (ADL powder)				
		#27	#28	#29	#30	#83	#27	#28	#29	#30	#31
Total colony count (cfu/g)	NEN-EN-ISO 4833 (ML/AL/42)	3,250	9,000	4,000	3,250	2,000	540	330	690	740	520
***Bacillus cereus* (cfu/g)**	ISO 7932 NEN 6875 (ML/AL/21)	< 10	< 40	< 40	< 10	10	< 10	< 10	< 10	< 40	< 40
Enterobacteriaceae (cfu/g)	NEN-ISO 21528-2 (ML/AL/55)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
***Escherichia coli* (cfu/g)**	NEN-ISO 16649-2 (ML/AL/41)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
***Listeria monocytogenes* in 25 g (cfu/g)**	NEN-EN-ISO 11290-1 (ML/AL/15)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
***Salmonella* spp. in 25 g (cfu/g)**	NEN-EN-ISO 6579 (ML/AL/61)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
***Staphylococcus aureus* (cfu/g)**	NEN-EN-ISO 6888-1 (ML/AL/60)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
***Clostridium perfringens* (cfu/g)**	ISO 7937 (ML/AL/35)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Yeasts (cfu/g)	NEN-ISO 21527-1 (ML/AL/62), ISO 7954:1987	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Moulds (cfu/g)	NEN-ISO 21527-1 (ML/AL/62), ISO 7954:1987	< 10	< 10	< 10	< 10	< 10	< 10	< 40	< 10	< 10	< 10

ISO: International Organization for Standardization.

Information was provided on the accreditation of the laboratories that conducted the analyses presented in the application.

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

3.4.1. Stability

The applicant performed stability tests with several independently produced batches of the NF forms. ADL frozen and ADL paste are to be stored in the dark, at −18°C, with RH 20–40%, in closed package, with an intended shelf-life of 5 months. ADL dried and ADL powder are to be stored in the dark, at 5°C, with RH 20–40%, in closed package, with an intended shelf-life of 6 months. Regarding the ADL frozen and ADL paste, the stability tests were carried out at the standard storage conditions, for a period of time that covers or exceeds the proposed shelf-life, for a limited number of batches and time points. The microbiological profile of ADL frozen and ADL paste during storage (Table 7), as well as the free fatty acids (ISO 660, Cold solvent method) and the water activity of ADL paste (Table 8) were reported.

Table 7: Microbiological stability of ADL frozen under standard storage conditions

Parameter (unit)	Batch number (ADL frozen)					
	#21	#112	#117	#112	#117	#21
Time (months)	**0**			**7**	**10**	**13**
Total colony count (cfu/g)	7,300	3,000	3,900	< 4,000	1,100	2,400
***Bacillus cereus* (cfu/g)**	< 10	< 10	< 10	< 10	< 10	< 10
Enterobacteriaceae (cfu/g)	< 10	< 10	40	< 10	< 10	< 10
***Escherichia coli* (cfu/g)**	< 10	< 10	< 10	< 10	< 10	< 10
***Listeria monocytogenes* in 25 g (cfu/g)**	ND	ND	ND	ND	ND	ND
***Salmonella* spp. in 25 g (cfu/g)**	ND	ND	ND	ND	ND	ND
***Staphylococcus aureus* (cfu/g)**	< 10	< 10	< 10	< 10	< 10	< 10
***Clostridium perfringens* (cfu/g)**	< 10	< 10	< 10	< 10	< 10	< 10
Yeasts (cfu/g)	< 10	< 10	< 10	< 40	< 40	< 10
Moulds (cfu/g)	< 10	< 10	< 10	< 40	< 40	< 10

ND: not detected.

Table 8: Microbiological stability of ADL paste under standard storage conditions

Parameter (unit)	Batch number (ADL paste)														
	#89	#92	#95	#98	#101	#90	#93	#97	#100	#103	#91	#94	#96	#99	#102
Time (months)	0					5									
Total colony count (cfu/g)	7,300	4,100	4,600	19,000	12,000	890	840	880	920	9,000	/	/	/	/	/
***Bacillus cereus* (cfu/g)**	10	10	10	10	10	< 10	< 10	< 10	< 10	< 10	/	/	/	/	/
Enterobacteriaceae (cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 40	/	/	/	/	/
***Escherichia coli* (cfu/g)**	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	/	/	/	/	/
***Listeria monocytogenes* in 25 g (cfu/g)**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	/	/	/	/	/
***Salmonella* spp. in 25 g (cfu/g)**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	/	/	/	/	/
***Staphylococcus aureus* (cfu/g)**	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	/	/	/	/	/
***Clostridium perfringens* (cfu/g)**	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	/	/	/	/	/
Yeasts (cfu/g)	< 10	< 10	10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	/	/	/	/	/
Moulds (cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	/	/	/	/	/

Parameter (unit)	Batch number (ADL paste)														
	#89	#92	#95	#98	#101	#90	#93	#97	#100	#103	#91	#94	#96	#99	#102
aw	0.992	0.993	0.992	0.992	0.993	0.983	0.993	0.983	0.989	0.981	0.997	0.995	0.991	0.990	0.993
FFA in the extracted oil (% w/w)	1.98	2.22	2.57	2.17	2.31	/	/	/	/	/	4.80	5.04	4.45	4.33	4.92

/: not provided.
ND: not detected.

Regarding ADL dried and ADL powder, the microbiological stability testing was carried out at the standard storage conditions, for a period of time that covers or exceeds the proposed shelf-life of 6 months, for a limited number of batches and time points (Table 9).

Table 9: Microbiological stability of ADL dried/ADL powder under standard storage conditions

Parameter (unit)	Batch number (ADL dried)		Batch number (ADL powder)			Batch number (ADL dried)		Batch number (ADL powder)		
	#84	#85	#86	#87	#68	#84	#85	#86	#87	#68
Time (months)			0			12	12	23	24	24
Total colony count (cfu/g)	7,100	32,000	41,000	25,000	66,000	5,500	2,600	/	5,000	3,000
Bacillus cereus (cfu/g)	/	/	< 100	< 100	< 100	/	/	< 10	< 10	< 10
Enterobacteriaceae (cfu/g)	< 100	< 100	< 100	< 100	50	< 100	< 100	< 10	< 10	< 10
Escherichia coli (cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Listeria monocytogenes in 25 g (cfu/g)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella spp. in 25 g (cfu/g)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Staphylococcus aureus (cfu/g)	< 100	< 100	< 100	< 100	< 100	< 100	< 100	ND	< 10	< 10
Clostridium perfringens (cfu/g)	/	/	< 10	< 10	< 10	/	/	< 10	< 10	< 10
Yeasts (cfu/g)	/	/	< 10	< 10	< 10	/	/	ND	< 10	< 10
Moulds (cfu/g)	/	/	< 10	< 10	< 10	/	/	ND	< 10	< 10
aw	/	/	/	/	/	/	/	0.547	0.549	0.397

/: not provided.
ND: not detected.

Moreover, the applicant provided data on the PV values of five batches of ADL powder, stored under normal conditions for a period between 14 and 16 months. PV values for t = 0 were not provided (Table 10).

Table 10: Oxidative stability of ADL powder under standard storage conditions

Parameter	Batch number (ADL powder)					Analytical method
	#72	#73	#74	#75	#76	
Time (months)	14–16					
Peroxide value (meq O_2/kg fat)	0.9	3.8	< 0.2	4.7	7.7	EN ISO 3960

meq: milliequivalents.

Furthermore, the applicant investigated the oxidative stability of the NF (peroxide value: EN ISO 3960; p-anisidine: NEN-EN-ISO 6885:2000), free fatty acid content, microbiological profile and histamine (ELISA) in five batches of the ADL powder, stored under accelerated conditions (37°C), for a

period of 30 days, and attempted to extrapolate the results to t = 6 months under normal storage conditions (Table 11).

Table 11: Microbiological stability and oxidative stability of fat of ADL powder, under accelerated storage conditions

Parameter (unit)	Batch number (ADL powder)									
	#27	#28	#29	#30	#31	#27	#28	#29	#30	#31
Time (months)	0					30 days–37°C				
aw	/	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	/
FFA as oleic acid (% in fat)	/	1.5	4.0	3.6	0.7	1.4	5.2	4.7	1.6	/
Peroxide value (meq O_2/kg fat)	/	2.5	2.3	1.1	2.3	9.2	4.0	3.3	7.1	/
p-Anisidine value	/	0.0	1.6	3.1	6.7	5.5	1.7	0.7	4.1	/
Total colony count (cfu/g)	540	330	690	740	520	310	< 4,000	950	580	< 4,000
***Bacillus cereus* (cfu/g)**	< 10	< 10	< 10	< 40	< 40	< 10	< 10	< 10	< 10	< 10
Enterobacteriaceae (cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
***Escherichia coli* (cfu/g)**	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
***Listeria monocytogenes* in 25 g (cfu/g)**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
***Salmonella* spp. in 25 g (cfu/g)**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
***Staphylococcus aureus* (cfu/g)**	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
***Clostridium perfringens* (cfu/g)**	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Yeasts (cfu/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Moulds (cfu/g)	< 10	< 40	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Histamine (mg/kg)	15	19	14	16	14	< 2.5	< 2.5	< 2.5	< 2.5	2.9

cfu: colony forming units; FFA: free fatty acids; meq: milliequivalents; ND: not detected; /: not provided.

The Panel notes that there are limitations regarding the stability data provided. The samples analysed at t = 0 were not always the same NF batches analysed at later time points. For some of the NF forms, the number of batches analysed at different time points during storage is not adequate (low). Furthermore, the Panel notes that the selected time points do not always offer the possibility to conclude on the stability of all NF forms for the intended shelf-life. Nevertheless, the Panel observes that the values of most of the analysed batches do not exceed the given specification limits.

Regarding the stability study performed under accelerated conditions for the ADL powder (Table 11), fluctuations were observed among the PV values of the analysed batches at t = 30 days, with some of the values being above the respective specification limit, indicating oxidation of fats. Additionally, there is no consistency regarding the p-anisidine values of the analysed batches at t = 30 days. The accelerated stability studies provided by the applicant cannot sufficiently predict the microbiological stability of the NF due to limitations in the study design. Nevertheless, the Panel notes that the microbiological values in ADL powder at the end of the accelerated stability study are low, despite the elevated temperature (37°C). The Panel concludes that the water activity values in the NF at the beginning and at the end of the accelerated stability study are low, acting as a deterrent to the microbiological growth in the NF.

Due to the limitations of the stability data provided, 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, the stability data do not raise safety concerns.

Stability in the intended for use matrices

Since the NF is going to be used as an ingredient in other food products, EFSA asked the applicant to investigate the stability when the NF is used as an ingredient in the intended-for-use matrices (see

Section 3.7.2 Proposed uses and use levels). The applicant investigated the lipid hydrolysis and oxidation of products containing the NF as an ingredient (cereal bars with ADL powder, patties with ADL paste, tomato soup with ADL powder and mushroom soup with ADL powder) after manufacture and after different duration of storage (Table 12).

The cereal bars with the NF (ADL cereal bars) comprised 11% of ADL powder and a mixture of cereals and cereal derivatives, dried fruits and seed oil. The patties with the NF (ADL patties) comprised 60% of ADL paste and a mixture of vegetables, rapeseed oil and seasonings. The ADL patties were deep-fried (180°C for t = 3 min), oven cooked (200°C for 20 min before packaging and storage (7°C), with an intended shelf-life of 21 days. To prepare the ADL tomato soups, the applicant added ADL powder to commercial soups at a quantity equal to 10% w/v and adjusted the pH of the final formulations using HCl. Subsequently, the soups were sterilised (121°C, 20 min). ADL mushroom soups were prepared in a way similar to the ADL tomato soups.

The applicant reported the concentration of heterocyclic aromatic amines (HCAs), polycyclic aromatic hydrocarbons (PAHs), acrylamide, chloropropanols (2-MCPD and 3-MCPD), dioxins and furans after the manufacture of the products. The applicant tested one composite sample per food item (which consisted of 24 samples of ADL cereal bars, 11 samples of ADL patties and 12 samples of ADL soups). The applicant reported that for the ADL powder used, the concentration of acrylamide was 147 μg/kg, FFA was 2.18 g/100 g, PV was 1.9 meq O_2/kg and HCAs, 2-MCPD, 3-MCPD and PAHs were below the respective LODs. Any change regarding processing-generated contaminants due to the use of NF as an ingredient in the intended-for-use matrices could not be assessed due to the absence of proper control samples (ambiguous manufacturing process of control samples).

Table 12: Analytical data on food products containing the NF as an ingredient

Parameter (unit)	ADL cereal bar (20°C)[1]		ADL patty (7°C)[1]		ADL tomato soup (20°C)[1]		ADL mushroom soup (20°C)[1]	
Time	**0 days**	**6 months**	**0 days**	**21 days**	**0 days**	**6 months**	**0 days**	**6 months**
pH	/	/	6.53	6.53	4.45	4.46	6.72	6.02
aw	0.608	0.638	0.981	0.987	0.992	0.976	1	0.981
Peroxide value (meq O_2/kg fat)	15.7	8.3	1.6	2.9	/	3.9	1.8	14.3
Free Fatty acids (g/100g)	4.80	7.25	0.53	0.35	1.99	4.50	1.06	8.68

1: Results from one composite sample per food item tested (which consisted of 24 samples of ADL cereal bars, 11 samples of ADL patties, and 12 samples of ADL soups); /: not provided.

The applicant performed microbiological analyses of the food products described above containing the NF as an ingredient. The Panel notes that the microbiological values reported in the ADL-containing products do not raise safety concerns.

The Panel further notes that the food items containing the NF have to comply with existing legislative limits, such as microbiological levels set in Regulation (EC) 2073/2005 and the benchmark levels of acrylamide in bakery products established by Regulation (EU) No 2017/2158.

The stability data on microbial contamination or lipid hydrolysis and oxidation in food matrices tested did not raise safety concern. Provided that the specifications are met also at the end of the shelf-life, and that products containing the NF are compliant with respective legislative limits on processing-generated contaminants, the stability data do not raise safety concerns.

3.5. Specifications

The specifications of the NF are indicated in Table 13.

Table 13: Specifications of the NF

Description:
ADL frozen: whole, blanched and frozen *A. diaperinus* larva
ADL paste: whole, blanched, ground and frozen *A. diaperinus* larva
ADL dried: whole, blanched and freeze-dried *A. diaperinus* larva
ADL powder: whole, blanched, freeze-dried and ground *A. diaperinus* larva (powder)

Parameters	Unit	ADL frozen	ADL paste	ADL dried	ADL powder
Appearance	–	Brown/light brown larvae	Brown/light brown paste	Light-brown dried larvae	Light-brown powder
Moisture	% w/w	65–80	65–80	1–5	1–5
Crude protein (N × 6.25)	% w/w	12–25	12–25	50–70	50–70
Fat	% w/w	5–12	5–12	20–35	20–35
Digestible carbohydrates	% w/w	0.4–2	0.4–2	1.5–3.5	1.5–3.5
Dietary fibre	% w/w	1–4	1-4	3–6	3–6
Chitin*	% w/w	≤ 2.6	≤ 2.6	≤ 9.1	≤ 9.1
Ash	% w/w	≤ 1.5	≤ 1.5	≤ 5	≤ 5
Peroxide value	meq O_2/kg fat	≤ 0.2	≤ 0.2	≤ 5	≤ 5
Heavy metals					
Lead	mg/kg	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1
Cadmium	mg/kg	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05
Minerals					
Copper	mg/kg	≤ 6.6	≤ 6.6	≤ 23	≤ 23
Manganese	mg/kg	≤ 3	≤ 3	≤ 6.5	≤ 6.5
Zinc	mg/kg	≤ 47	≤ 47	≤ 147	≤ 147
Mycotoxins					
Aflatoxin B1	µg/kg	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1
Aflatoxins (Sum of B1, B2, G1, G2)	µg/kg	≤ 0.4	≤ 0.4	≤ 0.4	≤ 0.4
Deoxynivalenol	µg/kg	≤ 40	≤ 40	≤ 40	≤ 40
Ochratoxin A	µg/kg	≤ 0.4	≤ 0.4	≤ 0.4	≤ 0.4
Microbiological					
Total aerobic colony count	cfu/g	≤ 10^5	≤ 10^5	≤ 10^5	≤ 10^5
Enterobacteriaceae	cfu/g	≤ 100	≤ 100	≤ 100	≤ 100
Escherichia coli	cfu/g	≤ 50	≤ 50	≤ 50	≤ 50
Listeria monocytogenes	in 25 g	Not detected	Not detected	Not detected	Not detected
Salmonella spp.	in 25 g	Not detected	Not detected	Not detected	Not detected
Bacillus cereus	cfu/g	≤ 100	≤ 100	≤ 100	≤ 100
Coagulase-positive staphylococci	cfu/g	≤ 100	≤ 100	≤ 100	≤ 100
Sulfite-reducing anaerobes	cfu/g	≤ 30	≤ 30	≤ 30	≤ 30
Yeasts and Moulds	cfu/g	≤ 100	≤ 100	≤ 100	≤ 100

cfu: colony forming units.
*: Chitin calculated as ADF (ANAL-10351).

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

There is no documented history of safe consumption of the NF or its source, i.e. *A. diaperinus* larvae. According to the applicant, food products containing *A. diaperinus* larvae have been marketed in certain countries of the European Union for up to ~ 15 years, but precise consumption figures were not made available.

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

The target population proposed by the applicant is the general population, except for food supplements, for which the target population proposed by the applicant is the adult population.

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[4] hierarchy, and the maximum use levels are reported in Table 14. In addition, the applicant intends to market the NF (ADL powder) for use in food supplements, at a maximum dose of 4 g per day.

Table 14: Food categories and maximum use levels intended by the applicant

FoodEx2 level	FoodEx2 code	Food category	Maximum use levels (g NF/100 g)			
			ADL frozen	ADL paste	ADL powder	ADL dried
3	A00EY	Cereal bars	/	/	25	25
4	A02PN	Whey powder	/	/	35	/
3	A04LK	Processed and mixed breakfast cereals	/	/	10	10
4	A00EN	Porridge (dry, to be diluted)	/	/	15	/
4	A005K	Bread and rolls with special ingredients added	/	/	20	/
5	A03ZB	Sandwich with processed meat topping/filling	/	/	20	/
5	A03ZG	Sandwich with meat and vegetable topping/filling	/	/	20	/
4	A0CSK	Pre-mixes (dry) for baked products	/	/	10	/
5	A007L	Dried pasta	/	/	10	/
5	A007Y	Dried stuffed pasta	28	28	10	/
5	A007R	Asian-style noodles other than glass Noodles	/	/	10	/
3	A03TE	Meat imitates	40	40	15	/
5	A04GP	Bovine and pig, minced meat	14	14	5	/
5	A049S	Bovine, minced meat	14	14	5	/
4	A01RG	Pig fresh meat	14	14	5	/
5	A03XF	Meat burger (no sandwich)	14	14	5	/
5	A03XG	Meat balls	14	14	5	/
2	A024F	Sausages	14	14	5	/
4	A03TH	Milk imitates	/	/	10	/
4	A03TQ	Dairy imitates other than milk	/	/	10	/
3	A0B9J	Soups (dry mixture uncooked)	/	/	15	/
4	A03ZN	Pizza and pizza-like dishes	/	/	5	5
3	A040 M	Pasta and rice (or other cereal)-based dishes	/	/	5	/
3	A06HL	Snacks other than chips and similar	/	/	10	10
3	A005Y	Crackers and breadsticks	/	/	10	/
4	A0EQX	Chips/crisps	/	/	10	/
4	A0EQS	Chocolate/cocoa-based products	/	/	5	/
5	A01BN	Peanut butter	/	/	15	/

/: The NF form is not intended to be used.

[4] FoodEx2 is an EFSA standardised food classification and description system https://www.efsa.europa.eu/en/data/data-standardisation

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 14), 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 an mg/kg body weight (bw) basis), among the EU dietary surveys, are presented in Table 15.

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 15: Intake estimate of the NF resulting from its use 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[(a)]	Highest[(a)]	Lowest[(b)]	Highest[(b)]
Infants	< 1	29	204	115	889
Young children[(c)]	1 to < 3	153	417	345	1,089
Other children	3 to < 10	148	428	290	997
Adolescents	10 to < 18	48	311	124	700
Adults[(d)]	≥ 18	37	174	80	508

(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 08/04/2022. 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 08/04/2022. The lowest and the highest P95 intake observed among all EU surveys are reported in these columns (P95 intake 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.

Regarding the intake of the NF in the form of food supplements, the target population is limited to adults and the proposed maximum daily dose of the NF is 4 g per day. Taking into consideration the default body weight of 70 kg for adults (EFSA Scientific Committee, 2012), this corresponds to a total intake of 565 mg/kg bw per day, considering the highest P95 intake from the NF used as an ingredient, for adults.

Table 16: Total intake of the NF resulting from the uses of the NF as an ingredient and as a food supplement

Population group	Age (years)	Body weight[(a)] (kg)	Highest[(b)] P95 intake from the NF used as an ingredient (mg/kg bw per day)	Intake from the NF used as a food supplement (mg/kg bw per day)[(c)]	Total intake[(d)] (mg/kg bw per day)
Infants	< 1	5	889	0	889
Toddlers	1 to < 3	12	1,089	0	1,089
Other children	3 to < 10	23.1	997	0	997
Adolescents	10 to < 18	43.4 or 61.3	700	0	700
Adults	≥ 18	70	508[(e)]	57	565

(a): Default and average body weights are defined in EFSA Scientific committee (2012).
(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database. The highest P95 intake observed among all surveys is reported in this column (P95 intake calculated based on less than 60 individuals is not considered).
(c): Intake in 'mg/kg bw per day' is calculated by considering the use levels in 'mg/day' and default body weights defined in EFSA Scientific Committee (2012).
(d): Total intake is the sum of the intake from NF ingredient use (highest P95 intake) and from the NF used as a food supplement, for each population group.
(e): Intakes are assessed separately for adults [18–65 years], elderly [65–75 years] and very elderly (≥ 75 years); the maximum intake among these three subpopulations is reported here.

3.7.4. Estimate of exposure to undesirable substances

Based on the highest P95 intake estimate (Table 16), EFSA estimated exposure to undesirable substances (heavy metals, toxins) from the NF for all population groups. The specification limits

(Table 13) were used as maximum concentrations of the undesirable substances. When specification limits for a substance of possible concern have not been proposed, the maximum values reported for the analysed batches were used. 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. The assessment of the intake of manganese (Mn) from the NF is provided in Section 3.9 Nutritional information.

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

No ADME data have been provided for the NF. The NF forms are mainly composed of protein, carbohydrates and fat which are normal constituents of the human diet. The Panel considers the constituents of the NF are expected to undergo normal metabolic processes.

3.9. Nutritional information

The applicant provided nutritional analysis of the NF. The NF consists mainly of protein, fat, digestible carbohydrates, dietary fibre (mainly chitin) and inorganic matter. Due to the higher water content in the undried NF forms compared to the dried ones, the concentrations of these components are lower in ADL frozen and ADL paste compared to ADL dried and ADL powder. Analytical data on the amino acid composition, the fatty acid content, minerals and vitamins have been provided for several batches of the NF forms.

Protein content and protein quality

The NF contains on average 18.5 (± 1.3) g crude protein per 100 g ADL frozen/ADL paste, and 61.0 (± 1.3) g crude protein per 100 g ADL dried/ADL powder, calculated using a nitrogen-to-protein conversion factor of 6.25. The Panel notes that the use of this conversion factor overestimates the level of true protein content in the lesser mealworm due to the presence of non-protein nitrogen in chitin (Janssen et al., 2017b). Based on the amino acid profile of the insects, Janssen et al. (2017b) determined a conversion factor of 4.86 for lesser mealworm. Using this factor, the protein content of the NF amounts to 14.4 g/100 g in ADL paste/ADL frozen and 47.5 g/100 g in ADL dried/ADL powder. The applicant, based on the amino acid profile of the NF and following a methodological approach similar to the one presented by Janssen et al. (2017b), determined a conversion factor of 5.0 (the applicant rounded the initially determined value of 5.04), the use of which results in a protein content of 14.8 g/100 g in ADL paste and 48.8 g/100 g in ADL dried/ADL powder. For regulatory purposes for nutritional labelling, protein is defined as the total nitrogen measured by the Kjeldahl method multiplied by a nitrogen-to-protein conversion factor of 6.25 [Regulation (EU) No 1169/2011 on the provision of food information to consumers].

The applicant quantified the content of amino acids in five batches of the ADL paste, four batches of ADL dried and one batch of ADL powder according to ISO13903:2005 and Commission Regulation (EC) No 152/2009 (Appendix A), and all essential amino acids were found to be present.

In addition, the applicant conducted a study of the true ileal digestibility during transit through a dynamic computer-controlled *in vitro* model of the stomach and small intestine (tiny-TIM) (Unpublished study report, 2022). The ADL powder was the NF form tested. Casein was used as a reference protein. The tests were conducted by an accredited laboratory in accordance with GLP. The true ileal protein digestibility was expressed as percentage of the bio-accessible nitrogen from the NF, i.e. calculated using the nitrogen-to-protein conversion factor of 5 estimated by the applicant. The true ileal digestibility was higher for casein (86.7 ± 0.6%) compared to ADL powder (75.8 ± 1.9%), indicating that the protein of ADL powder is less bio-accessible than casein. Following the recommendation by FAO (2013), the protein quality was determined by the 'Digestible Indispensable Amino Acid Score (DIAAS)'.

Based on the FAO indispensable amino acid requirements for the older child, adolescent and adult using the protein conversion factor of 6.25, the resulting DIAAS for protein from ADL powder corresponded to 58 ± 3%. However, the use of 6.25 as conversion factor overestimates the amount of protein, leading consequently to a lower DIAAS value since it also considers the non-protein nitrogen. When using the nitrogen-to-protein conversion factor of 5, as estimated by the applicant for the true protein, the DIAAS for protein from ADL powder corresponded to 75.8 ± 4%, compared to the DIAAS for casein of 82 ± 4%. The first limiting amino acids, both for casein and ADL powder, were the sulfur-containing ones (methionine and cysteine).

Considering quality of the crude protein (Nx6.25), if the NF entirely replaces other protein sources of higher quality, it might negatively impact protein nutrition if the overall protein intake is low. Based on the high (95th percentile) intake levels of the NF (Section 3.4, Table 16) with a maximum crude protein content of ~ 61% (ADL dried, ADL powder) (Section 3.4, Table 2), the corresponding protein intake per kg bw per day from the NF would amount up to 0.54 g for infants, 0.81 g for young children, 0.66 g for other children, 0.44 for adolescents and 0.34 g for adults. These intakes correspond to up to 41%, 90%, 78%, 53% and 42% of the respective dietary reference values (PRIs) (EFSA NDA Panel, 2012) for protein for infants, young children, other children, adolescents and adults.

The Panel notes that the applicant calculated also the DIAAS value considering in the calculations the conversion factor of 5 (DIAAS = 75.8 ± 4%), which indicates that the quality of the 'true protein' could be higher compared to the quality of crude protein.

Taking into account that the NF would 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.

Fatty acids, vitamins and minerals

The major fatty acids in the NF are oleic acid, linoleic acid and palmitic acid (Appendix B). On average, saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids constitute 34.5 ± 1.6%, 33.2 ± 2.1% and 32.4 ± 2.3% of the total fatty acids, respectively (ISO 12966-2/4). The average trans-fatty acid content is 0.8 ± 0.3% of total fatty acids.

The applicant provided analytical data on the levels of some minerals and vitamins (Tables 17–19).

Table 17: Minerals in the NF, analysed by ICP-MS

Minerals (mg/100 g)	Batch number									
	ADL paste					ADL dried				ADL powder
	#63	#64	#65	#66	#67	#43	#46	#47	#51	#58
Calcium	21	14	16	15	17	56	49	50	57	49
Copper	0.64	0.52	0.48	0.51	0.54	2.2	1.8	1.8	1.8	1.8
Iron	1.6	1.4	1.3	1.3	1.4	5.0	4.6	5.0	5.6	5.3
Magnesium	33	35	29	29	34	110	110	110	130	120
Manganese	0.24	0.19	0.21	0.21	0.22	0.52	0.58	0.59	0.64	0.6
Phosphorus	210	220	170	190	190	760	730	770	750	790
Potassium	260	270	190	210	220	960	950	980	970	980
Sodium	56	54	45	43	52	200	210	210	230	220
Zinc	3.5	4.0	3.7	4.3	4.3	12	13	13	14	14

Table 18: Vitamins in the undried forms NF

Vitamins	Analytical method	Batch number							
		ADL paste					ADL frozen		
		#16	#63	#64	#66	#67	#113	#114	#115
Biotin (mg/100g)	SPR	0.012	0.037	2.027	0.015	0.027	0.049	0.047	0.053
Cyanocobalamine (µg/100g)	J. AOAC 2008, vol 91 No 4/LC-UV/DAD	1.52	0.487	< 0.25	0.361	0.576	0.753	0.908	0.749
Folate (mg/100g)	AOAC 2013.13 and NMKL 111:1985/ Nephelometry	0.082	0.054	0.063	0217	0.06	< 0.5	< 0.5	< 0.5
Niacin (mg/100g)	EN 15652:2009/ LC-FLD	2.83	3.55	3.94	2.62	2.3	4.31	4.27	4.48

Vitamins	Analytical method	Batch number							
		ADL paste					ADL frozen		
		#16	#63	#64	#66	#67	#113	#114	#115
Pantothenic acid (mg/100g)	AOAC 2012.16/ LC-MS	0.95	1.13	1.13	1.02	0.77	1.95	1.9	2.16
Pyridoxine (mg/ 100g)	EN 14164/LC-FLD	0.16	0.10	0.10	0.44	0.25	0.067	0.068	0.09
Riboflavin (mg/ 100g)	EN 14152:2003, mod./LC-FLD	0.33	0.20	0.22	0.44	0.29	0.1	0.087	0.088
Thiamin base (mg/100g)	EN 14122:2003, mod./LC-FLD	0.33	0.59	0.812	0.077	0.69	/	/	/
Thiamin.HCl (mg/100g)	EN 14122:2003, mod./LC-FLD	0.042	0.074	0.104	0.001	0.088	/	/	/
Retinol (µg/ 100 g)	EN 12823-1 2014/ LC-DAD	< 21	< 21	< 21	< 21	< 21	/	/	/
Cholecalciferol (µg/100 g)	EN 12821:2009/ LC-DAD	< 0.25	< 0.25	0.539	< 0.25	0.477	/	/	/
alpha-Tocopherol (mg/100 g)	EN 12822:2014/ LC-FLD	0.495	0.54	0.45	0.374	0.461	0.525	0.576	0.734

SPR: Surface plasmon resonance.
LC-DAD = Liquid Chromatography - Diode-Array Detection.
LC-FLD = Liquid chromatography - Fluorescence Detection.
(LC-MS = Liquid Chromatography - Mass Spectrometry.
LC-UV/DAD = Liquid Chromatography-Ultraviolet-Diode-Array Detection.
/: not provided.

Table 19: Vitamins in the dried forms of the NF

Vitamins	Analytical method	ADL dried				ADL powder
		#43	#46	#47	#51	#58
Biotin (mg/kg)	SPR	0.044	0.052	0.051	0.044	0.058
Cyanocobalamine (µg/100 g)	J. AOAC 2008, vol 91 No 4/LC-UV/ DAD	4.78	3.15	3.76	2.37	0.663
Folate (mg/kg)	AOAC 2013.13 and NMKL 111:1985/Nephelometry	2.5	3.29	2.64	1.96	2.53
Niacin (mg/100 g)	EN 15652:2009/LC-FLD	11.4	11.6	11.9	11.7	14.1
Pantothenic acid (mg/kg)	AOAC 2012.16/LC-MS	4.01	4015	3.97	5006	4.03
Pyridoxine (mg/kg)	EN 14164/LC-FLD	0.54	0.48	0.49	0.44	0.16
Riboflavin (mg/kg)	EN 14152:2003, mod./LC-FLD	1.49	1.17	1.36	1.4	1.09
Thiamin base (mg/kg)	EN 14122:2003, mod./LC-FLD	0.188	0.175	0.182	0.192	0.228
Thiamin.HCl (mg/kg)	EN 14122:2003, mod./LC-FLD	0.238	0.223	0.213	0.243	0.290
Retinol (µg/100 g)	EN 12823-1 2014/LC-DAD	< 21	< 21	< 21	< 21	< 21
Cholecalciferol (µg/100 g)	EN 12821:2009/LC-DAD	1.08	1.11	1.99	1.36	2.3
alpha-Tocopherol (mg/100 g)	EN 12822:2014/LC-FLD	< 0.08	1.53	1.43	1.58	0.705

SPR: Surface plasmon resonance.
LC-DAD = Liquid Chromatography - Diode-Array Detection.
LC-FLD = Liquid chromatography - Fluorescence Detection.
(LC-MS = Liquid Chromatography - Mass Spectrometry.
LC-UV/DAD = Liquid Chromatography-Ultraviolet-Diode-Array Detection.

Considering the mean concentrations reported in Table 17–19 and the estimated P95 of exposure to the NF (Table 16), the Panel notes that none of the existing upper levels for the analysed micronutrients are expected to be exceeded, for any population group.

Intake of Mn, for which upper levels have not been established by EFSA, was also considered. 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 Panel, 2006).

The concentration of Mn in the NF (ADL dried/ADL powder), according to specifications (Table 13), may reach 6.5 mg/kg. This concentration is comparable 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). EFSA estimated the intake of Mn from the NF, considering the product specification for Mn (Table 13) and the estimated daily intake of the NF for all population groups (Table 15). Results are presented in Table 20.

Table 20: Intake estimate of Mn resulting from the use of the NF under the proposed uses and at the maximum proposed use levels, both as ingredient and food supplement

Population group	Age (years)	Mean intake (mg/day)		P95 intake (mg/day)	
		Lowest[(a)]	Highest[(a)]	Lowest[(b)]	Highest[(b)]
Infants	< 1	0.00	0.01	0.00	0.03
Young children[(c)]	1 to < 3	0.01	0.03	0.03	0.08
Other children	3 to < 10	0.02	0.06	0.04	0.15
Adolescents	10 to < 18	0.02	0.13	0.05	0.28
Adults[(d)]	≥ 18	0.02	0.08	0.04	0.26

Mn: manganese; NF: novel food.
(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 8/4/2022. 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 08/04/2022. The lowest and the highest P95 intake observed among all EU surveys are reported in these columns (P95 intake 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 P95 intake of Mn from the NF ranges from 0.03 mg/day in infants to 0.28 mg/day in adolescents. As compared to the highest mean background Mn intake estimates, the additional intake of manganese from the NF would be 0.8% for infants, 2.4% for young children, 4.3% for other children, 4.6% for adolescents and 4.3% in adults. The Panel considers that such an increase of Mn intake (< 5% of the highest mean background intake)[5] from the NF is not of concern.

Antinutritional factors

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 2.2 g

[5] 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' judgment 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'.

chitin in 100 g of ADL paste and 7.8 g of chitin in 100 g of ADL dried (Table 3). 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 negatively affecting their bioavailability, as reported for dietary fibres in general (Baye et al., 2017).

Insects may contain antinutritional factors (ANFs) such as tannins, oxalates, phytates, hydrogen cyanide (Shantibala et al., 2014; Meyer-Rochow et al., 2021), thiaminases (Nishimune et al., 2000) and protease inhibitors (Eguchi, 1993). The applicant determined the concentrations of total polyphenols in ADL paste and ADL powder (five batches per form) (Table 21), phytic acid, trypsin inhibitor, hydrocyanic acid, oxalic acid and tannins in ADL dried (three batches), as well as tannins in ADL dried (three batches) (Table 22). 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 21: Polyphenol content in the NF (ADL paste, ADL powder)

Parameter (unit)	Analytical method	ADL paste					ADL powder				
		#55	#63	#64	#65	#66	#54	#58	#60	#62	#68
Total polyphenols (g GAE/100 g)	Folin–Ciocalteu assay	0.3	0.2	0.3	0.3	0.3	0.7	0.7	0.8	0.8	0.5

Table 22: Antinutritional factors in the NF (ADL powder, ADL dried)

Parameter (unit)	Analytical method	ADL powder			ADL dried		
		#35	#33	#34	#109	#110	#111
Phytic acid (mg/100 g)	ANAL-10445	959	817	959	/	/	/
Trypsin inhibitor (mg/100 g)	ISO 14902	87	58	30	/	/	/
Hydrocyanic acid (mg/kg)	EN-16160 (modified)	< 1	< 1	< 1	/	/	/
Oxalic acid (g/kg)	Suppressed Conductivity and Charge Detection	0.192	0.174	0.199	/	/	/
Tannins (%)	Folin-Denis	/	/	/	0.86	0.86	0.86

/: not provided.

Overall, 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

Production of defensive secretions (comprising mainly benzoquinones) by *A. diaperinus* adults (beetles) has been reported (Hassemer et al., 2015; Tschinkel, 1975; Tseng et al., 1971). However, no evidence that these compounds are produced by *A. diaperinus* larvae has been found. Upon EFSA's request, the applicant investigated the occurrence of benzoquinones (1,4-benzoquinone; 2-methyl-1,4-benzoquinone; 2-ethyl-1,4-benzoquinone) in the NF (five batches of ADL powder) via an in-house UPLC-FLD and the results were below the LOD (< 0.6 mg/kg) for each compound. A full description of the implemented method as well as the results of the respective validation procedures have been provided. Considering the information above, the Panel notes that *A. diaperinus* larvae should be reared separately from the adults.

Regarding the safety of chitin present in the NF, the applicant referred to the EFSA 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 the lesser mealworm. As reviewed by Komi et al. (2018), chitin has been shown to activate a variety of innate (eosinophils, macrophages) and adaptive immune cells (IL-4/IL-13 expressing T helper type-2 lymphocytes) and this implies the potential to promote hypersensitivity. The limited information

on potential adverse effects of chitin based on available literature is inconclusive as previously discussed by the Panel (EFSA NDA Panel, 2021a,b).

No toxicological studies on the NF or its source were retrieved in a literature search, neither by the applicant nor by EFSA. Following EFSA's request, the applicant provided a 90-day oral toxicity study in rodents. This study is described in Section '3.10.2 Subchronic toxicity'.

3.10.1. Genotoxicity

No genotoxicity studies with the NF as testing material have been submitted by the applicant or retrieved from the literature. The Panel considers that, given the nature of the NF, there are no concerns with regard to genotoxicity.

3.10.2. Subchronic toxicity

The applicant provided a subchronic 90-day toxicity study in Wistar Han rats given the NF (ADL powder) for 13 weeks at dose levels of 0, 3, 10 and 20% of the feed. The diets of each group were nutritionally balanced to ensure similar amounts of macronutrients. The study was claimed proprietary by the applicant and was conducted in compliance with OECD principles of GLP and in accordance with OECD test guideline No 408 (OECD, 1998).

Statistically significant findings are indicated in Appendix C.

Haematological analysis revealed statistically significant decreases in white blood cells, lymphocytes and large unstained cells, while reticulocytes were increased. The findings were not clearly dose related and fall in the range of the historical controls. Therefore, the changes are not considered as adverse by the Panel.

At histopathological examination, the presence of multifocal macrophage aggregates (of minimal or mild degree) in the mesenteric lymph nodes was noted in six out of 10 males administered the highest dose, in one of the females of the low- and one female of the mid-dose, as well as in all females administered the highest dose. The findings were not accompanied by inflammation, necrosis or alteration of the nodal architecture of the lymph nodes. The Panel considered the observed effect as a non-adverse immunological adaptation to the NF.

No test item-related or toxicologically relevant changes were noted in any of the remaining parameters investigated in this study (i.e. mortality, clinical appearance, body weight, food consumption, functional observation, ophthalmoscopy, clinical laboratory investigations, macroscopic examination and organ weights).

The Panel considered that the highest dose tested (20% NF in the feed) is the no observed adverse effect level (NOAEL) of this study, corresponding to a mean NF (ADL powder) intake of 12,469 mg/kg bw per day for male rats and 15,302 mg/kg bw per day for female rats.

3.10.3. Human data

The applicant referred to two published studies involving human subjects and using lesser mealworm-derived products as test material (Vangsoe et al., 2018a,b). The Panel considers that the test material is not representative of the NF and notes that these studies were not designed to assess safety. Therefore, no conclusions can be drawn from these studies on the safety of the NF.

3.11. Allergenicity

The *Tenebrionidae* mealworm family belongs to the 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). The currently available literature on food allergy related to insects is scarce and the few prevalence studies available are mainly for Asian populations (China and Laos) (Ji et al., 2009; Barennes et al., 2015).

Primary sensitisation to insect species belonging to the same family as the lesser mealworm, the source of the NF, has been recently investigated in humans and animals (EFSA NDA Panel, 2021a,b). Leni et al. (2020), using a shotgun bottom-up proteomic approach, identified actin, myosin and tropomyosin to be among the most abundant proteins in lesser mealworm. Performing an *in silico* allergenicity assessment, tropomyosin was identified as the prevalent potential allergen in lesser mealworm.

Cross-reactivity of lesser mealworm proteins to other allergens has been reported. In the study of Leni et al. (2020), peptides of lesser mealworm had high sequence homology with other insects, crustaceans and mites. The tropomyosin of lesser mealworm reacted with IgE serum of a patient allergic to the tropomyosin of crustacean, confirming the cross-reactivity to crustaceans. IgE serum from patients allergic to crustaceans or house dust mites showed cross-reactivity to proteins of lesser mealworms which had undergone different processing (raw, boiled, lyophilised, fried) (van Broekhoven et al., 2016). Patients allergic to shrimp were found to be at allergic risk due to lesser mealworm consumption also by Broekman et al. (2017).

A frequently reported cause of allergic reaction to insects, including *A. diaperinus,* relates to occupational exposure (skin contact and inhalation). Occupational allergy to *A. diaperinus* has been reported by Schroeckenstein et al. (1988) in three workers in the USA, confirmed by skin prick tests (for larvae, pupae and adults of *A. diaperinus*).

Additional aspects should be taken into consideration depending on the feed substrate used to rear the source of the NF, as it might include common allergenic foods (Mancini et al., 2020). The applicant reported that a substrate with gluten-containing grains is used. ADL frozen and ADL dried were analysed for gluten, which was detected and quantified only in ADL dried. The Panel notes that changes in the feed can possibly introduce additional allergens (e.g. gluten), including allergens which require mandatory labelling according to Annex II of Regulation (EU) No 1169/2011, in the NF, since traces of the allergens may remain in the gut of insects despite the fasting step implemented (Mancini et al., 2020).

The Panel considers that the consumption of the NF may trigger primary sensitisation to lesser mealworm proteins. The Panel also considers that allergic reactions may occur in subjects allergic to crustaceans and dust mites (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 comprises frozen and dried formulations of the lesser mealworm (*Alphitobius diaperinus* larva) as whole, and in the form of paste or powder. The production process is sufficiently described and does not raise safety concerns and the Panel considers that the NF is sufficiently characterised. ADL dried and ADL powder are concentrates of, respectively, ADL frozen and ADL paste. Apart from water (in ADL frozen or paste), the main components of the NF are protein and fat besides smaller amounts of digestible carbohydrates and chitin. The concentrations of contaminants in the NF depend mainly on the occurrence of such substances in the insect feed. Provided that the respective 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. Moreover, the applicant intends to market the NF as a food supplement, with adults being the target population. Intake was estimated based on the use of the NF as an ingredient in the intended food categories at the maximum proposed levels and for adults also as a food supplement. The highest intake estimate (based on the scenario of inclusion of the highest amount of NF dry matter) was calculated for young children (1–< 3years old) ranging from 345 to 1,326 mg NF/kg bw per day at the 95th percentile. 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 the population to the analysed undesirable substances (heavy metals, mycotoxins).

The Panel notes that 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 protein digestibility of the NF (ADL dried) is 75.8 ± 1.9%, with a DIAAS value of 58 ± 3.0% when calculating the protein content using 6.25 as nitrogen-to-protein conversion factor, and a DIAAS value of 73 ± 4.0% when calculating the protein content using the nitrogen-to-protein conversion factor calculated by the applicant (5) as compared to casein (82 ± 4%). The first limiting amino acids were the sulfur-containing ones. Considering that the NF will not be the sole source of dietary protein and disintegrated 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 exceeded considering the proposed uses and use levels. The reported values for the levels of 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 insoluble and 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 reduce 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.

There is no documented history of safe consumption of the NF or its source, i.e. *A. diaperinus* larvae. Upon EFSA's request, the applicant submitted a subchronic 90-day toxicity study, the results of which did not raise safety concerns. No adverse effects were observed in the study up to the highest dose tested, corresponding to a mean NF (ADL powder) intake of 12,469 mg/kg bw per day for male rats and 15,302 mg/kg bw per day for female rats, which the Panel considers as the NOAEL of the study.

Taking into account the compositional characterisation, the production process, the nature of the NF, as well as that no adverse health effects have been observed up to the highest dose level tested in the subchronic toxicity study, the Panel considers that, despite the lowest resulting Margin of Exposure (MoE) being 11.4, the NF does not raise safety concerns under the proposed conditions of use. Additionally, the Panel considers that there are no other safety concerns, provided the larvae are reared separately from the adults. The Panel considers that the consumption of the NF may induce primary sensitisation and allergic reactions to lesser mealworm proteins and may cause allergic reactions in subjects with allergy to crustaceans and dust mites due to cross-reactivity. Additionally, the Panel notes that allergens from the feed (e.g. gluten) may end up in the NF.

5. Conclusions

The Panel concludes that the NF, frozen and dried forms of whole *A. diaperinus* larva, is safe under the proposed conditions of use.

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

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the data claimed as proprietary by the applicant (compositional data, stability studies, *in vitro* protein digestibility study, subchronic 90-day toxicity study).

6. Recommendation

The Panel recommends that research is undertaken on the allergenicity to lesser mealworm, including cross-reactivity to other allergens.

7. Steps taken by EFSA

1) On 17 July 2018 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of whole and ground lesser mealworm (*Alphitobius diaperinus*) larvae products as novel food. Ref. Ares (2018)3788147 – 17/7/2018.
2) On 17 July 2018, a valid application on whole and ground lesser mealworm (*Alphitobius diaperinus*) larvae products, which was submitted by Proti-Farm Holding NV (currently "Ynsect NL B.V."), was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2018/0125) and the scientific evaluation procedure was initiated.
3) On 20 November 2018, 12 February 2019, 29 May 2019, 21 August 2019, 4 September 2019, 9 January 2020, 11 June 2020, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 7 February 2019, 22 February 2019, 21 August 2019 (x2), 8 January 2020, 10 June 2020, 31 March 2022 additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) On 19 April 2022, EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of frozen and freeze-dried formulations of the lesser mealworm (*Alphitobius diaperinus* larva) as a novel food. Ref. Ares (2022)3080791 – 19/4/2022.
6) During its meeting on 26 April 2022, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of frozen and freeze-dried formulations of the lesser mealworm (*Alphitobius diaperinus* larva) as a NF pursuant to Regulation (EU) 2015/2283.

References

Aalbu RL, Triplehorn CA, Campbell JM, Brown KW, Somerby RA and Thomas DB, 2002. Family 106. Tenebrionidae Latreille 1802. In: Arnett RH, Thomas MC, Skelley PE and Frank JH (eds.). American Beetles. Volume 2. Polyphaga: Scarabaeoidea through Curculionoidea. CRC Press, Boca Raton FL. pp. 463–509.

Alborzi AR and Rahbar A, 2012. Introducing Alphitobius diaperinus, (Insecta: Tenebrionidae) as a new intermediate host of Hadjelia truncata (Nematoda). Iranian Journal of Parasitology, 7, 92.

Anastopoulos I, Bhatnagar A, Bikiaris DN and Kyzas GZ, 2017. Chitin adsorbents for toxic metals: a review. International Journal of Molecular Sciences, 18, 114.

Barennes H, Phimmasane M and Rajaonarivo C, 2015. Insect consumption to address undernutrition, a national survey on the prevalence of insect consumption among adults and vendors in Laos. PLoS One, 10, e0136458.

Baye K, Guyot JP and Mouquet-Rivier C, 2017. The unresolved role of dietary fibers on mineral absorption. Critical Reviews in Food Science and Nutrition, 57, 949–957.

Bednarska AJ and Świątek Z, 2016. Subcellular partitioning of cadmium and zinc in mealworm beetle (Tenebriomolitor) larvae exposed to metal-contaminated flour. Ecotoxicology and Environmental Safety, 133, 82–89.

Bertola M and Mutinelli F, 2021. A systematic review on viruses in mass-reared edible insect species. Viruses, 13, 2280.

Binder M, Mahler V, Hayek B, Sperr WR, Schöller M, Prozell S, Wiedermann G, Valent P, Valenta R and Duchêne M, 2001. Molecular and immunological characterization of arginine kinase from the Indian meal moth, Plodia interpunctella, a novel cross-reactive invertebrate pan-allergen. The Journal of Immunology, 167, 5470–5477.

Broekman HC, Knulst AC, de Jong G, Gaspari M, den Hartog Jager CF, Houben GF and Verhoeckx KC, 2017. Is mealworm or shrimp allergy indicative for food allergy to insects? Molecular Nutrition & Food Research, 61, 1601061.

De las Casas E, Harein PK and Pomeroy BS, 1972. Bacteria and fungi within the lesser mealworm collected from poultry brooder houses. Environmental Entomology, 1, 27–30.

Dibusz K and Vejvodova P, 2020. Systematic literature search to assist EFSA in the preparatory work for the safety assessment of novel food applications and traditional food notifications. EFSA supporting publication 2020:EN-1774, 72 pp. https://doi.org/10.2903/sp.efsa.2019.EN-1774

Dunford JC and Kaufman PE, 2006. Lesser mealworm, litter beetle, Alphitobius diaperinus (Panzer) (Insecta: Coleoptera: Tenebrionidae). EDIS, 2006.

EFSA (European Food Safety Authority), 2011. Use of the EFSA comprehensive European food consumption database in exposure assessment. EFSA Journal 2011;9(3):2097, 50 pp. https://doi.org/10.2903/j.efsa.2011.2097

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2011. Scientific Opinion on Scientific Opinion on risk based control of biogenic amine formation in fermented foods. EFSA Journal 2011;9(10):2393, 93 pp. https://doi.org/10.2903/j.efsa.2011.2393

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2019. Evaluation of the health risks related to the presence of cyanogenic glycosides in foods other than raw apricot kernels. EFSA Journal 2019;17(4),5662, 45 pp. https://doi.org/10.2903/j.efsa.2019.5662

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2010. Scientific Opinion on the safety of "Chitin-Glucan" as a Novel Food ingredient. EFSA Journal, 8(7):1687, 17. https://doi.org/10.2903/j.efsa.2010.1687

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2012. Scientific opinion on dietary reference values for protein. EFSA Journal 2012;10(2):2557, 58 pp. https://doi.org/10.2903/j.efsa.2012.2557

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2013. Scientific Opinion on Dietary Reference Values for manganese. EFSA Journal 2013;11(11):3419, 44 pp. https://doi.org/10.2903/j.efsa.2013.3419

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), Turck D, Bresson J-L, Burlingame B, Dean T, Fairweather-Tait S, Heinonen M, Hirsch-Ernst KI, Mangelsdorf I, McArdle H, Naska A, Neuhäuser-Berthold M, Nowicka G, Pentieva K, Sanz Y, Siani A, Sjödin A, Stern M, Tomé D, Vinceti M, Willatts P, Engel K-H, Marchelli R, Pöting A, Poulsen M, Salminen S, Schlatter J, Arcella D, Gelbmann W, de Sesmaisons-Lecarré A, Verhagen H and van Loveren H, 2016. Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283. EFSA Journal 2016;14(11):4594, 24 pp. https://doi.org/10.2903/j.efsa.2016.4594

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck D, Castenmiller J, De Henauw S, Hirsch-Ernst KI, Kearney J, Maciuk A, Mangelsdorf I, McArdle HJ, Naska A, Pelaez C, Pentieva K, Siani A, Thies F, Tsabouri S, Vinceti M, Cubadda F, Frenzel T, Heinonen M, Marchelli R, Neuhäuser-Berthold M, Poulsen M, Prieto Maradona M, Schlatter JR, van Loveren H, Ververis E and Knutsen HK, 2021a. Scientific Opinion on the safety of dried yellow mealworm (Tenebrio molitor larva) as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2021;19(1):6343, 29 pp. https://doi.org/10.2903/j.efsa.2021.6343

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), Turck D, Bresson J-L, Burlingame B, Dean T, Fairweather-Tait S, Heinonen M, Hirsch-Ernst KI, Mangelsdorf I, McArdle H, Naska A, Neuhäuser-Berthold M, Nowicka G, Pentieva K, Sanz Y, Siani A, Sjödin A, Stern M, Tomé D, Vinceti M, Willatts P, Engel K-H, Marchelli R, Pöting A, Poulsen M, Salminen S, Schlatter J, Arcella D, Gelbmann W, de Sesmaisons-Lecarré A, Verhagen H and van Loveren H, 2021b. Scientific Opinion on the safety of frozen and dried formulations from whole yellow mealworm (Tenebrio molitor larva) as a novel food pursuant to Regulation (EU)2015/2283. EFSA Journal 2021;19(8):6778, 30 pp. https://doi.org/10.2903/j.efsa.2021.6778

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck D, Castenmiller J, De Henauw S, Hirsch-Ernst KI, Kearney J, Maciuk A, Mangelsdorf I, McArdle HJ, Naska A, Pelaez C, Pentieva K, Siani A, Thies F, Tsabouri S, Vinceti M, Cubadda F, Frenzel T, Heinonen M, Marchelli R, Neuhäuser-Berthold M, Poulsen M, Maradona MP, Schlatter JR, van Loveren H, Azzollini D and Knutsen HK, 2021c. Scientific Opinion on the safety of frozen and dried formulations from migratory locust (Locusta migratoria) as a Novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2021;19(7):6667, 30 pp. https://doi.org/10.2903/j.efsa.2021.6667

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck D, Bohn T, Castenmiller J, De Henauw S, Hirsch-Ernst KI, Maciuk A, Mangelsdorf I, McArdle HJ, Naska A, Pelaez C, Pentieva K, Siani A, Thies F, Tsabouri S, Vinceti M, Cubadda F, Frenzel T, Heinonen M, Marchelli R, Neuhäuser-Berthold M, Poulsen M, Prieto Maradona M, Schlatter JR, van Loveren H, Goumperis T and Knutsen HK, 2021d. Scientific Opinion on the safety of frozen and dried formulations from whole house crickets (Acheta domesticus) as a Novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2021;19(8):6779, 29 pp. https://doi.org/10.2903/j.efsa.2021.6779

EFSA Scientific Committee, 2011. Guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. EFSA Journal 2011;9(12):2438, 21 pp. https://doi.org/10.2903/j.efsa.2011.2438

EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 45 pp. https://doi.org/10.2903/j.efsa.2012.2579

EFSA Scientific Committee, 2015. Risk profile related to production and consumption of insects as food and feed. EFSA Journal 2015;13(10):4257, 65 pp. https://doi.org/10.2903/j.efsa.2015.4257

Eguchi M, 1993. Protein protease inhibitors in insects and comparison with mammalian inhibitors. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 105, 449–456.

Elowni EE and Elbihari S, 1979. Natural and experimental infection of the beetle, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) with *Choanotaenia infundibulum* and other chicken tapeworms. Veterinary Science Communications, 3, 171–173.

FAO (Food and Agriculture Organization of the United Nations), 2013. Dietary protein quality evaluation in human nutrition, Report of an FAO Expert Consultation, FAO, Rome, Italy.

van der Fels-Klerx HJ, Camenzuli L, Van Der Lee MK and Oonincx DGAB, 2016. Uptake of cadmium, lead and arsenic by Tenebrio molitor and Hermetia illucens from contaminated substrates. PLoS One, 11.

Finke MD, 2007. Estimate of chitin in raw whole insects. Zoo Biology: Published in Affiliation with the American Zoo and Aquarium Association, 26, 105–115.

Francisco O and do Prado AP, 2001. Characterization of the larval stages of Alphitobius diaperinus (Panzer) (Coleoptera: Tenebrionidae) using head capsule width. Revista Brasileira De Biologia, 61, 125–131.

Franco LDO, Maia RDCC, Porto ALF, Messias AS, Fukushima K and Campos-Takaki GMD, 2004. Heavy metal biosorption by chitin and chitosan isolated from Cunninghamella elegans (IFM 46109). Brazilian Journal of Microbiology, 35, 243–247.

Galindo PA, Lombardero M, Borja J, Gomez E, Feo F, Barber D and Garcia R, 2001. A New Arthropod panallergen? Allergy, 56, 195–197.

Geden CJ and Hogsette J, 1994. Research and extension needs for integrated pest management for arthropods of veterinary importance.

Ghannem S, Touaylia S and Boumaiza M, 2018. Beetles (Insecta: Coleoptera) as bioindicators of the assessment of environmental pollution. Human and Ecological Risk Assessment: an International Journal, 24, 456–464.

Gupta YP, 1987. Anti-nutritional and toxic factors in food legumes: a review. Plant Foods for Human Nutrition, 37, 201–228.

Hassemer MJ, Sant'Ana J, De Oliveira MWM, Borges M, Laumann RA, Caumo M and Blassioli-Moraes MC, 2015. Chemical composition of Alphitobius diaperinus (Coleoptera: Tenebrionidae) abdominal glands and the influence of 1, 4-benzoquinones on its behavior. Journal of Economic Entomology, 108, 2107–2116.

Holmes RP and Kennedy M, 2000. Estimation of the oxalate content of foods and daily oxalate intake. Kidney International, 57, 1662–1667.

Hosen M, Khan AR and Hossain M, 2004. Growth and development of the lesser mealworm, Alphitobius diaperinus (Panzer) (Coleoptera: Tenebrionidae) on cereal flours. Pakistan Journal of Biological Sciences, 7, 1505–1508.

Houbraken M, Spranghers T, De Clercq P, Cooreman-Algoed M, Couchement T, De Clercq G, Verbeke S and Spanoghe P, 2016. Pesticide contamination of Tenebrio molitor (Coleoptera: Tenebrionidae) for human consumption. Food Chemistry, 201, 264–269.

Huber K, Gouilloud L and Zenner L, 2007. A preliminary study of natural and experimental infection of the lesser mealworm Alphitobius Diaperinus (Coleoptera: Tenebrionidae) with Histomonas Meleagridis (Protozoa: Sarcomastigophora). Avian Pathology, 36, 279–282. https://doi.org/10.1080/03079450701447317

Janssen RH, Lakemond CMM, Fogliano V, Renzone G, Scaloni A and Vincken J-P, 2017a. Involvement of phenoloxidase in browning during grinding of Tenebrio molitor larvae. PLoS One, 12.

Janssen RH, Vincken JP, van den Broek LA, Fogliano V and Lakemond CM, 2017b. Nitrogen-to-protein conversion factors for three edible insects: tenebrio molitor, Alphitobius diaperinus, and Hermetia illucens. Journal of Agricultural and Food Chemistry, 65, 2275–2278.

Ji K, Chen J, Li M, Liu Z, Wang C, Zhan Z, Wu X and Xia Q, 2009. Anaphylactic shock and lethal anaphylaxis caused by food consumption in China. Trends in Food Science & Technology, 20, 227–231.

Karunamoorthy G, Chellappa DJ and Anandan R, 1994. The life history of Subulura brumpti in the beetle Alphitobius diaperinus. Indian Veterinary Journal, 71, 12–15.

Komi DEA, Sharma L and Cruz CSD, 2018. Chitin and its effects on inflammatory and immune responses. Clinical Reviews in Allergy and Immunology, 54, 213–223.

Kurečka M, Kulma M, Petříčková D, Plachý V and Kouřimská L, 2021. Larvae and pupae of Alphitobius diaperinus as promising protein alternatives. European Food Research and Technology, 247, 2527–2532.

Lambkin TA, 2001. Investigations into the management of the darkling beetle, Rural Industries Research and Development Corporation, Kingston, Australia. 99.

Leni G, Tedeschi T, Faccini A, Pratesi F, Folli C, Puxeddu I, Migliorini P, Gianotten N, Jacobs J, Depraetere S, Caligiani A and Sforza S, 2020. Shotgun proteomics, in-silico evaluation and immunoblotting assays for allergenicity assessment of lesser mealworm, black soldier fly and their protein hydrolysates. Scientific Reports, 10, 1–10.

Lindqvist L and Block M, 1995. Excretion of cadmium during moulting and metamorphosis in Tenebrio molitor (Coleoptera; Tenebrionidae). Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology andEndocrinology, 111, 325–328.

Mancini S, Fratini F, Tuccinardi T, Degl'Innocenti C and Paci G, 2020. Tenebrio molitor reared on different substrates: is it gluten free? Food Control, 110, 107014.

Meyer-Rochow VB, Gahukar RT, Ghosh S and Jung C, 2021. Chemical composition, nutrient quality and acceptability of edible insects are affected by species, developmental stage, gender, diet, and processing method. Foods, 10, 1036.

Muzzarelli RAA, 1973. Natural chelating polymers. Alginic acid, chitin and chitosan.

Muzzarelli RAA, Boudrant J, Meyer D, Manno N, DeMarchis M and Paoletti MG, 2012. Current views on fungal chitin/chitosan, human chitinases, food preservation, glucans, pectins and inulin: a tribute to Henri Braconnot, precursor of the carbohydrate polymers science, on the chitin bicentennial. Carbohydrate Polymers, 87, 995–1012.

Nappi AJ and Christensen BM, 2005. Melanogenesis and associated cytotoxic reactions: applications to insect innate immunity. Insect Biochemistry and Molecular Biology, 35.

Nappi AJ and Ottaviani E, 2000. Cytotoxicity and cytotoxic molecules in invertebrates. BioEssays, 22, 469–480. https://doi.org/10.1002/(SICI)1521-1878(200005)22:5%3c469:AID-BIES9%3e3.0.CO;2-4

Nappi AJ and Vass E, 1993. Melanogenesis and the generation of cytotoxic molecules during insect cellular immune-reactions. Pigment Cell Research, 6, 117–126. https://doi.org/10.1111/j.1600-0749.1993.tb00590.x

Nishimune T, Watanabe Y, Okazaki H and Akai H, 2000. Thiamin is decomposed due to Anaphe spp. Entomophagy in seasonal ataxia patients in Nigeria. The Journal of Nutrition, 130, 1625–1628.

OECD (Organisation for Economic Co-operation and Development), 1998a. Test No. 408: Repeated dose 90-day oral toxicity study in rodents. In: OECD guidelines for the testing of chemicals, Section 4: Health effects, 10.

Oonincx DG, Van Broekhoven S, Van Huis A and van Loon JJ, 2015. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. PLoS One, 10.

Panzer G, 1797. Faunae Insectorum Germanicae initia oder Deutschlands Insecten. Vol. 47. Place Felsecker, Norimbergae, Felsecker, Norimbergae.

Paoletti MG, Norberto L, Cozzarini E and Musumeci S, 2009. Role of Chitinases in human stomach for chitin digestion: AMCase in the gastric digestion of chitin and chit in gastric pathologies.

Poma G, Cuykx M, Amato E, Calaprice C, Focant JF and Covaci A, 2017. Evaluation of hazardous chemicals in edible insects and insect-based food intended for human consumption. Food and Chemical Toxicology, 100, 70–79.

Rao BSN and Prabhavathi T, 1982. Tannin content of foods commonly consumed in India and its influence on ionisable iron. Journal of the Science of Food and Agriculture, 33, 89–96.

Reese G, Ayuso R and Lehrer SB, 1999. Tropomyosin: an invertebrate pan–allergen. International Archives of Allergy and Immunology, 119, 247–258.

Roberts GA, 1992. Chitin chemistry, Macmillan International Higher Education.

Rumbos CI, Karapanagiotidis IT, Mente E and Athanassiou CG, 2018. The lesser mealworm Alphitobius diaperinus: a noxious pest or a promising nutrient source? Reviews in Aquaculture, 11, 1418–1437.

Rumpold BA and Schlüter OK, 2013. Nutritional composition and safety aspects of edible insects. Molecular Nutrition and Food Research, 57, 802–823.

SCF (Scientific Committee on Food), 2000. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of manganese. SCF/CS/NUT/UPPLEV/21 Final, 11 pp.
SCF/NDA Panel (Scientific Committee on Food, Panel on Dietetic Products, Nutrition and Allergies), 2006. Tolerable upper intake levels for vitamins and minerals. Available online: https://www.efsa.europa.eu/sites/default/files/efsa_rep/blobserver_assets/ndatolerableuil.pdf
Schlemmer U, Frølich W, Prieto RM and Grases F, 2009. Phytate in foods and significance for humans: food sources, intake, processing, bioavailability, protective role and analysis. Molecular Nutrition and Food Research, 53, S330–S375.
Schroeckenstein DC, Meier-Davis S, Graziano FM, Falomo A and Bush RK, 1988. Occupational sensitivity to Atphitabius diaperinus (Panzer) (lesser mealworm). Journal of Allergy and Clinical Immunology, 82, 1081–1088.
Shantibala T, Lokeshwari RK and Debaraj H, 2014. Nutritional and antinutritional composition of the five species of aquatic edible insects consumed in Manipur, India. Journal of Insect Science, 14.
Smith R, Hauck R, Macklin K, Price S, Dormitorio T and Wang C, 2021. A review of the lesser mealworm beetle (Alphitobius diaperinus) as a reservoir for poultry bacterial pathogens and antimicrobial resistance. World's Poultry Science Journal, 1–18.
Spilman TJ, 1991. 11, Darkling Beetles (Tenebrionidae, Coleoptera). In Insect and Mite Pests in Food. (Gorham JR, ed). United States Department of Agriculture, Agricultural Handbook 655, 185–214, 589–598.
Sugumaran M, Nellaiappan K and Valivittan K, 2000. A new mechanism for the control of phenoloxidase activity: inhibition and complex formation with quinone isomerase. Archives of Biochemistry and Biophysics, 379, 252–260.
Tschinkel WR, 1975. A comparative study of the chemical defensive system of tenebrionid beetles: chemistry of the secretions. Journal of Insect Physiology, 21, 753–783.
Tseng YCL, Davidson JA and Menzer RE, 1971. Morphology and chemistry of the odoriferous gland of the lesser mealworm, Alphitobius diaperinus (Coleoptera: Tenebrionidae). Annals of the Entomological Society of America, 64, 425–430.
Unpublished study report, 2022 (amended). Digestion of protein from larvae of *Alphitobius diaperinus* during transit through a dynamic computer controlled *in vitro* model of the stomach- and small intestine (tiny-TIM), Project number: P22-249 as amendment of Study Report V21116.
Van Broekhoven S, Bastiaan-Net S, de Jong NW and Wichers HJ, 2016. Influence of processing and in vitro digestion on the allergic cross-reactivity of three mealworm species. Food Chemistry, 196, 1075–1083.
Vangsoe MT, Joergensen MS, Heckmann LHL and Hansen M, 2018. Effects of insect protein supplementation during resistance training on changes in muscle mass and strength in young men. Nutrients, 10, 335.
Vangsoe MT, Thogersen R, Bertram HC, Heckmann LHL and Hansen M, 2018. Ingestion of insect protein isolate enhances blood amino acid concentrations similar to soy protein in a human trial. Nutrients, 10, 1357.
Vijver M, Jager T, Posthuma L and Peijnenburg W, 2003. Metal uptake from soils and soil–sediment mixtures by larvae of Tenebrio molitor (L.) (Coleoptera). Ecotoxicology and Environmental Safety, 54, 277–289.
Zhao X, Li L, Kuang Z, Luo G and Li B, 2015. Proteomic and immunological identification of two new allergens from silkworm (Bombyx mori L.) pupae. Central-European. Journal of Immunology, 40, 30.

Abbreviations

2MCPD	3-Monochloropropane-1,2-Diol
3-MCPD	3-Monochloropropane-1,2-Diol
ADF	Acid Detergent Fibre
ADL	Acid Detergent Lignin
ADME	Absorption, Distribution, Metabolism and Excretion
ANF	Antinutritional Factors
AOAC	Association of Official Analytical Chemists
Aw	water activity
BIOHAZ	EFSA Panel on Biological Hazards
Bw	Body Weight
Cfu	Colony Forming Units
CLA	Conjugated Linoleic Acid
CONTAM	EFSA Panel on Contaminants in the Food Chain
DIAAS	Digestible Indispensable Amino Acid Score
DRVs	Dietary Reference Values
FAO	Food and Agriculture Organization of the United Nations
FFA	Free Fatty Acids
GC-MS/MS	Gas Chromatography with Tandem Mass Spectrometry
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practice

HACCP	Hazard Analysis Critical Control Points
HCAs	Heterocyclic Aromatic Amines
HPLC/UV	High Performance Liquid Chromatography/Ultraviolet detection
IAC-HPLC/UV	High Performance Liquid Chromatography with ultraviolet detection, with immunoaffinity column
IAC-LC/FLD	Liquid Chromatography with fluorescence detector, with immunoaffinity column
IAC-LC/UV	Liquid Chromatography with ultraviolet detection, with immunoaffinity column
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
ISO	International Organization for Standardization
LOD	Limit of Detection
LOQ	Limit of Quantification
meq	Milliequivalent
MRL	Maximum Residue Level
MUFA	Mono-Unsaturated Fatty Acids
N	nitrogen
ND	not detected
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NF	Novel Food
OECD	Organization for Economic Co-Operation and Development
PAHs	Polycyclic Aromatic Hydrocarbons
PRI	Population Reference Intakes
PUFA	Poly-Unsaturated Fatty Acids
PV	Peroxide Value
SFA	Saturated Fatty Acids

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

Amino acids (g/100 g NF)	ADL paste					ADL dried				ADL powder
	#15	#17	#18	#19	#20	#43	#46	#47	#51	#58
Alanine[1]	1.12	1.1	1.11	1.12	1.32	3.9	4.05	4.04	4.21	3.75
Arginine[1]	0.919	0.882	0.877	0.918	1.03	3.17	3.31	3.36	3.44	2.92
Aspartic acid[1]	1.48	1.43	1.45	1.52	1.68	5.42	5.43	5.59	5.67	4.75
Glutamic acid[1]	1.91	1.85	1.84	1.95	2.12	6.93	7.03	7.28	7.36	6.83
Glycine[1]	0.778	0.753	0.754	0.775	0.917	2.74	2.82	2.85	2.9	2.55
Histidine[1]*	0.584	0.558	0.571	0.582	0.665	1.98	2.03	2.04	2.16	1.87
Hydroxyproline[1]	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Isoleucine[1,*]	0.713	0.703	0.697	0.73	0.83	2.59	2.58	2.65	2.72	2.37
Leucine[1,*]	1.11	1.09	1.07	1.13	1.28	4.03	4.06	4.17	4.22	3.65
Lysine[1,*]	1.07	1.07	1.05	1.11	1.21	3.92	4.01	4.08	4.19	3.55
Ornithine[1]	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	0.2
Phenylalanine[1,*]	0.753	0.722	0.727	0.775	0.838	2.64	2.66	2.71	2.8	2.4
Proline[1]	1.05	1.04	1.05	1.07	1.21	3.47	3.6	3.63	3.76	3.48
Serine[1]	0.715	0.688	0.69	0.718	0.808	2.51	2.57	2.64	2.64	2.29
Threonine[1,*]	0.682	0.647	0.655	0.687	0.772	2.43	2.46	2.54	2.55	2.17
Tyrosine [1]	1.36	1.29	1.34	1.38	1.46	4.24	4.49	4.42	4.67	4.07
Valine[1,*]	0.966	0.943	0.938	0.98	1.12	3.38	3.44	3.47	3.61	3.12
Cysteine + **Cystine**[1]	0.137	0.145	0.144	0.133	0.157	0.524	0.539	0.555	0.547	0.551
Methionine[1,*]	0.221	0.243	0.23	0.226	0.26	0.808	0.866	0.878	0.843	0.827
Tryptophan (Total)[2,*]	0.202	0.206	0.205	0.203	0.234	0.698	0.72	0.736	0.754	0.718

1: ISO 13903:2005/IC-UV (Ion Chromatography-Ultraviolet detection).
2: EU 159/2009/IC-UV (Ion Chromatography-Ultraviolet detection).
*: Essential amino acids.

Appendix B – Detailed fatty acid profile analysis of the NF

Fatty acids (% of total fatty acids)	ADL frozen									ADL paste						ADL powder								
	#36	#38	#37	#57	#70	#71	#81	#80	#82	#18	#104	#105	#106	#107	#108	#77	#79	#78	#118	#53	#52	#41	#56	#39
C4:0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C6:0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C8:0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C10:0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C10:1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C11:0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C12:0	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1
C12:1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C13:0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C13:0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C13:0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C14:0 (iso)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C14:0	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.5	0.9	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.8	0.6	0.6	0.6	0.7	0.7	0.7	0.7
C14:1-n9t	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C14:1-n9c	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C15:0 (iso)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C15:0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C15:0	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
C15:1-n10t	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C15:1-n10c	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C16:0 (iso)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C16:0	27.1	25.9	26.0	24.9	23.3	23.9	24.3	24.7	24.2	26.1	26.0	26.2	25.8	25.9	24.1	25.8	30.6	26.4	25.0	25.0	26.0	26.5	26.2	25.2
C16:1-n9t	0.6	0.5	0.6	0.6	0.5	0.6	0.5	0.6	0.1	0.6	0.5	0.5	0.5	0.5	0.4	0.5	0.3	0.5	0.5	0.7	0.6	0.6	0.6	0.6
C16:1-n7t	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C16:1-n9c	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
C16:2n-4	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1	0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C16:3n-4	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C17:0 (anteiso)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C 17:0	0.3	0.4	0.4	0.5	0.4	0.4	0.4	0.5	0.4	0.5	0.3	0.4	0.4	0.4	0.3	0.4	0.4	0.5	0.4	0.5	0.4	0.5	0.4	0.5
C17:1-n7t	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C17:1-n7c	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C18:0	6.3	7.2	6.8	6.8	7.7	6.7	6.5	7.0	7.5	7.2	7.0	6.5	7.0	6.8	7.0	7.5	7.9	7.6	7.5	7.0	7.5	6.4	7.5	7.1
C18:1-n11T	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C18:1-n9T	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	0.1	< 0.1	0.1	0.1	< 0.1	< 0.1
C18:1-n7T	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.2	0.2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C18:1-n11c	0.7	0.8	0.7	0.8	0.5	0.4	0.4	0.4	0.5	0.9	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.0	0.9	0.9	0.9	1.0
C18:1-n9c	35.1	32.9	33.0	33.0	30.3	30.4	29.9	30.4	32.1	31.6	31.6	30.6	30.0	30.3	29.4	31.5	24.9	32.7	31.6	31.6	32.2	31.0	32.2	31.5
C18:1-n7c	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C18:1-n6c	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C18:2-5,9	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C18:2-n6T	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C18:2-n6c	26.1	27.9	33.0	29.3	34.0	34.1	34.5	33.2	30.3	28.9	30.0	31.2	31.6	31.4	34.1	30.2	30.9	28.5	31.0	29.7	28.0	29.7	27.7	29.4
9(Z), 11(E) C18:2 (CLA)	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.2	0.2	0.2	0.2	0.2
10(E), 12(Z) C18:2 (CLA)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C18:3-5,9,12	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C18:3n6	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C18:3n-4	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	0.1	0.1	0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C18:3 n3	1.5	1.8	1.6	1.6	1.7	1.8	1.7	1.5	2.0	1.6	1.8	2.0	2.1	2.0	2.0	1.7	2.1	1.6	1.8	1.7	1.8	1.9	1.8	1.9
C18:4-n3	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	0.1	0.1	0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C19:0	0.1	0.1	0.2	< 0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C19:1-n10t	0.2	0.2	0.2	0.3	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.3	0.3	0.3	0.3	0.3
C19:1-n7t	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C20:0	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.3	0.4	0.4

Fatty acids (% of total fatty acids)	ADL frozen									ADL paste						ADL powder								
	#36	#38	#37	#57	#70	#71	#81	#80	#82	#18	#104	#105	#106	#107	#108	#77	#79	#78	#118	#53	#52	#41	#56	#39
C20:2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
C20:3-5,11,14	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C20:3-n6t	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C20:3n6-cis-DGLA	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C20:3n3-cis-ETA	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C20:4 Omega 6	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C20:4 Omega 3	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C20:5n-3 (EPA)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C21:0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C22:0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C22:1-n11c	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C22:1-n9t	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C22:1-n9c	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C22:2-n6c	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C22:3, Omega 3	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C22:4-n6c	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C22:5n-6 (DPA)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C22:5n-3 (DPA)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C22:6n-3 (DHA)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C23:0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C24:0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
C24:1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
SFA	35.0	35.0	34.8	33.5	32.5	32.2	32.4	33.4	33.9	35.2	34.7	34.4	34.4	34.5	32.7	34.9	40.5	35.6	34.0	33.9	35.2	34.8	35.5	34.1
MUFA	37.2	35.1	35.2	35.4	31.7	31.9	31.4	31.8	33.6	34.0	33.2	32.2	31.6	31.8	30.9	33.1	26.5	34.3	33.1	34.4	34.7	33.4	34.8	34.3
PUFA	27.7	29.9	30.1	31.1	35.8	36.0	36.3	34.8	32.5	30.8	32.1	33.5	34.0	33.7	36.4	32.0	33.0	30.1	32.8	31.7	30.1	31.8	29.7	31.6
Trans FA %	1.0	0.9	1.0	1.2	0.5	0.6	0.6	0.6	0.5	1.0	0.6	0.6	0.5	0.5	0.5	0.6	0.6	0.7	0.6	1.2	1.2	1.1	1.2	1.2
Sum Omega 3 FA	1.5	1.8	1.6	1.6	1.7	1.8	1.7	1.5	2.0	1.6	1.8	2.0	2.1	2.1	2.0	1.7	2.1	1.6	1.8	1.7	1.8	1.9	1.8	1.9
Sum Omega 6 FA	26.1	28.0	28.3	29.3	34.0	34.2	34.6	33.3	30.4	29.0	30.1	31.3	31.7	31.5	34.2	30.3	30.9	28.5	31.0	29.8	28.1	1.1	27.8	29.5

Appendix C – Statistically significant findings of the 90-day toxicity study with the NF as testing material

Sex: Male		Reporting Haematology				Sex: Female		Reporting Haematology			
		WBC (10 /L) [G]	LYMPH (10 /L) [G]	LUC (10 /L) [G]	RETIC (10 /L) [G]			WBC (10^9/ L) [G]	LYMPH (10^9/ L) [G]	LUC (10^9/ L) [G]	RETIC (10^9/ L) [G]
0% Group 1	Mean	7.654	6.141	0.034	135.39	0% Group 1	Mean	3.87	3.245	0.029	137.93
	SD	1.394	1.006	0.013	19.72		SD	0.817	0.666	0.011	20.91
	N	10	10	10	10		N	10	10	10	10
3% Group 2	Mean	6.412	5.209	0.026	124.71	3% Group 2	Mean	2.822*	2.237*	0.013**	165.2
	SD	1.177	1.012	0.011	21.32		SD	0.776	0.835	0.008	35.65
	N	10	10	10	10		N	10	10	10	10
	tCtrl	0.84	0.85	0.76	0.92		tCtrl	0.73	0.69	0.45	1.2
10% Group 3	Mean	5.987 *	4.85	0.031	128.45	10% Group 3	Mean	2.915	2.292*	0.014**	136.68
	SD	1.352	1.181	0.013	22.29		SD	1.339	1.025	0.011	25.3
	N	10	10	10	10		N	10	10	10	10
	tCtrl	0.78	0.79	0.91	0.95		tCtrl	0.75	0.71	0.48	0.99
20% Group 4	Mean	6.848	5.619	0.033	128.38	20% Group 4	Mean	2.685*	2.056**	0.009**	181.30**
	SD	1.214	1.173	0.017	18.21		SD	0.588	0.502	0.006	37.1
	N	10	10	10	10		N	10	10	10	10
	tCtrl	0.89	0.91	0.97	0.95		tCtrl	0.69	0.63	0.31	1.31
Historical control	Mean	5.769	4.497	0.036	203.63	Historical control	Mean	3.823	3.139	0.022	221.62
	SD	1.745	1.515	0.024	49.78		SD	1.347	1.173	0.015	65.49
	N	178	178	178	178		N	176	176	176	176
* = $p \leq 0.05$; ** = $p \leq 0.01$						* = $p \leq 0.05$; ** = $p \leq 0.01$					

WBC: White Blood Cells; LYMPH: lymphocytes; LUC: large unstained cells; RETIC: reticulocytes.

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

Information provided in this annex is shown in an Excel file (downloadable at https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2022.7325#support-information-section).