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SCIENTIFIC OPINION

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Safety of frozen and dried forms of whole yellow mealworm (*Tenebrio molitor* larva) as a novel food pursuant to Regulation (EU) 2015/2283

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) | Dominique Turck | Torsten Bohn | Montaña Cámara | Jacqueline Castenmiller | Stefaan De Henauw | Karen Ildico Hirsch-Ernst | Ángeles Jos | Alexandre Maciuk | Inge Mangelsdorf | Breige McNulty | Androniki Naska | Kristina Pentieva | Alfonso Siani | Frank Thies | Margarita Aguilera-Gómez | Francesco Cubadda | Thomas Frenzel | Marina Heinonen | Monika Neuhäuser-Berthold | Helle Katrine Knutsen | Morten Poulsen | Miguel Prieto Maradona | Josef Rudolf Schlatter | Alexandros Siskos | Henk van Loveren | Gabriela Precup | Ermolaos Ververis | Harry J. McArdle

Correspondence: nif@efsa.europa.eu

The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

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 the safety of frozen, dried and powder forms of whole yellow mealworm (*Tenebrio molitor* larva) as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The term yellow mealworm refers to the larval form of the insect species *T. molitor*. The NF consists of the frozen and dried forms of the whole yellow mealworm. The frozen form mainly consists of water, crude protein, carbohydrates and fat, whereas the dried forms consist of crude protein, fat and carbohydrates. The Panel notes that the levels of contaminants in the NF highly depend on the occurrence levels of these substances in the insect feed. The Panel notes that there are no safety concerns regarding the stability of the NF if the NF complies with the proposed specification limits during its entire shelf life. The NF has a protein content that ranges between 13 and 48 g/100 g. The Panel acknowledges that the true protein content is overestimated when using the nitrogen-to-protein conversion factor of 6.25 due to the presence of non-protein nitrogen from chitin. The applicant proposed to use the NF as food ingredient in various food products. The target population proposed by the applicant is the general population. Considering the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous. The Panel notes that no safety concerns arise from the toxicological information of the NF. The Panel considers that the consumption of the NF might trigger primary sensitisation to yellow mealworm proteins and may cause allergic reactions in subjects allergic to crustaceans, dust mites and molluscs. Additionally, allergens from the feed may end up in the NF. The Panel notes that allergic reactions may occur upon consumption. The Panel concludes that the NF is safe under the proposed uses and use levels.

KEYWORDS

food safety, novel foods, *Tenebrio molitor* larva, yellow mealworm

CONTENTS

1 | INTRODUCTION

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

On 10 May 2018 the Belgian Insect Industry Federation (BiiF) submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283[1] to authorise placing on the market of frozen and dried forms of whole yellow mealworm (*Tenebrio molitor*) as a novel food (NF).

The target population is the general population. The application requests to authorise use of mealworm (*Tenebrio molitor*) in a number of foods/food categories.

On 2 December 2019 and in accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission asks the European Food Safety Authority to provide a scientific opinion on the safety of mealworm *(Tenebrio molitor)* as a novel food.

1.2 | Additional information

On 24 November 2020, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) adopted a scientific opinion on the safety of dried yellow mealworm (*Tenebrio molitor* larva) as NF pursuant to Regulation (EU) 2015/2283. The Panel concluded that the NF is safe for human consumption under the proposed uses and use levels (EFSA NDA Panel, 2021a). Following a favourable opinion of the Standing Committee on Plants, Animals, Food and Feed (Novel Food and Toxicological Safety section) on 22 April 2021, the European Commission adopted on 1 June 2021 Commission Implementing Regulation (EU) 2021/882[2] authorising the placing on the market of dried yellow mealworm as a NF under Regulation (EU) 2015/2283.

On 7 July 2021, the EFSA NDA Panel adopted a scientific opinion on the safety of frozen and dried formulations from whole yellow mealworm (*T. molitor* larva) as a novel food pursuant to Regulation (EU) 2015/2283. The Panel concluded that the NF is safe for human consumption under the proposed uses and use levels (EFSA NDA Panel, 2021b). Following a favourable opinion of the Standing Committee on Plants, Animals, Food and Feed (Novel Food and Toxicological Safety section) on 30 November 2021, the European Commission adopted on 8 February 2022 Commission Implementing Regulation (EU) 2022/169[3] authorising the placing on the market of frozen, dried and powder forms of yellow mealworm (*T. molitor* larva) as a NF under Regulation (EU) 2015/2283.

On 28 March 2023, the EFSA NDA Panel adopted a scientific opinion on the safety of UV-treated powder of whole yellow mealworm (*T. molitor* larva) as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The Panel concluded that the NF is safe for human consumption under the proposed uses and use levels (EFSA NDA Panel, 2023).

2 | DATA AND METHODOLOGIES

2.1 | Data

The safety assessment of this NF is based on data supplied in the application and information submitted by the applicant following EFSA's requests for supplementary information. 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 Commission Implementing Regulation (EU) 2017/2469.

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of a NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all 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 does not include a request for the protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283.

[1]Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/200. OJ L 327, 11.12.2015, pp. 1–22.

[2]Commission Implementing Regulation (EU) 2021/882 of 1 June 2021 authorising the placing on the market of dried *Tenebrio molitor* larva as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council, and amending Commission Implementing Regulation (EU) 2017/2470. OJ L 194/16, 2.6.2021.

[3]Commission Implementing Regulation (EU) 2022/169 of 8 February 2022 authorising the placing on the market of frozen, dried and powder forms of yellow mealworm (*Tenebrio molitor* larva) as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council, and amending Commission Implementing Regulation (EU) 2017/2470. OJ L 28, 9.2.2022, pp. 10–16.

2.2 | Methodologies

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

In the context of this opinion, EFSA's definition of dietary fibre (i.e. non-digestible carbohydrates plus lignin; EFSA NDA Panel, 2010) does not reflect the additional requirement of having a beneficial physiological effect demonstrated by generally accepted scientific evidence as laid down in Annex I of Regulation (EC) 1169/2011 for:

a. edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and,
b. edible synthetic carbohydrate polymers.

It is out of the scope of this opinion to establish whether the fraction of non-digestible carbohydrates present in the NF (chitin) meets the legal definition of dietary fibre in the EU or not.

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, which is the subject of the application, consists of frozen and dried forms of whole yellow mealworm (*T. molitor*), 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 of yellow mealworms. The frozen form mainly consists of water, crude protein, carbohydrates and fat, whereas the dried forms consist of crude protein, fat and carbohydrates. The NF is proposed to be used as whole frozen or whole dried insect, or in the form of powder, added as an ingredient to various food products such as soups, bakery products, pasta and confectionery, including chocolate. The NF will be added to foods intended for the general population.

3.2 | Identity of the NF

The NF comprises frozen and dried forms of whole yellow mealworm (*T. molitor*), whole or ground. The term 'mealworm' refers to the larval form of *T. molitor*, an insect species that belongs to the family of Tenebrionidae (darkling beetles). Another identified scientific synonym is *T. molitor* Linnaeus; 'yellow mealworm', 'mealworms', 'vers de farine', 'ténébrion meunier' and 'mealworm meal' are some of the common names for *T. molitor* larvae or products thereof.

The Eastern-Mediterranean region appears to be the point of origin for *T. molitor* sp. (Panagiotakopulu, 2000). However, *T. molitor* sp. is currently present in many regions worldwide, due to colonisation and trade (Panagiotakopulu, 2001). The applicant received the initial livestock of *T. molitor* that is used in the reproduction cycle from a third party and proceeded with the farming of the insects. The identity of the insects from the external supplier was established by using PCR testing.

The NF is intended to be marketed as (a) whole, blanched and frozen *T. molitor* larva (TM frozen), (b) whole, blanched and dried *T. molitor* larva (TM dried), (c) as powder of whole, blanched and dried *T. molitor* larva (TM powder). The larvae are farmed under controlled rearing conditions.

3.3 | Production process

According to the information provided, the NF is produced in line with Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Points (HACCP) principles. The production process can be divided into three distinctive parts, i.e. farming, harvesting and post-harvest processing.

Farming includes mating of the adult insect population and rearing of the larvae. The eggs are separated from the adult insects by sieving so that larvae can grow separately. After hatching from the eggs (10 days), the light yellow-brown larvae grow for 11 weeks in dedicated containers made of certified food contact material (polypropylene carbonate and polyethylene). The hard surface reduces the probability of plastic ingestion by the larvae (EFSA NDA Panel, 2021a, 2021b). The applicant stated that no pesticides, antimicrobial substances or veterinary medicinal products are used during the rearing of the larvae.

Yellow mealworms have the potential to bioaccumulate chemical agents such as heavy metals, pesticide residues and other undesirable compounds [e.g. polychlorinated biphenyls (PCBs), dioxins through their feed intake, Bednarska &

Swiaztek, 2016; Ghannem et al., 2018; Houbraken et al., 2016; Lindqvist & Block, 1995; Van der Fels-Klerx et al., 2016; Vijver et al., 2003]. The applicant reported that the feed administered to the insects is plant-derived and consists of materials such as vegetables (carrots) and cereal flour (wheat flour and bran) that follow the provisions of Regulation (EC) No 834/2007[4] and Commission Implementing Regulation (EU) 2021/1165,[5] and are compliant with Directive 2002/32/EC.

The applicant informed that the feed substrate used may contain gluten-containing grains and soy-derived ingredients. Water is administered to the larvae via the feed (vegetables).

During the rearing of the larvae, deceased insects and faeces are monitored and removed. Two distinct sorting steps are performed, when the larvae are ~7 and ~12 weeks old. Mechanical sieving separates the larvae from the substrate, exuvia and faeces. Deceased larvae have a darker colour compared to the alive larvae and are removed after visual inspection. The 7-week-old larvae are further grown, and the 12-week-old larvae are harvested to be processed. After the harvest (removal from the feed substrate), a 24-h fasting step is implemented, to allow the larvae to discard their bowel content. Deceased larvae after the fasting step are removed upon visual inspection.

The post-harvest processing includes the killing of the larvae by blanching in hot water (immersion for 1 to 5 min in boiling water), cooling down of the larvae and removal of the excess water. The blanching step contributes to the reduction of the microbial load of the larvae as well as elimination of potentially present viruses or parasites (Kooh et al., 2019; Vandeweyer et al., 2021). Furthermore, blanching reduces enzymatic activity (e.g. tyrosinase or phenoloxidase) (Janssen, Lakemond, et al., 2017) that might induce enzymatic browning in the larvae (Nappi & Christensen, 2005; Nappi & Ottaviani, 2000; Nappi & Vass, 1993; Sugumaran et al., 2000; Vigneron et al., 2014). The frozen form of the larvae is obtained by freezing the blanched larvae at −20°C to reach at least −18°C at the core of the insect in less than 5 h, followed by packaging and storage. The dried form is obtained by drying the blanched larvae in a ventilated oven at 65–70°C for 10.5–11 h (duration may vary depending on ambient conditions and volume of insects to be dried), resulting in a final product with water activity < 0.6. The dried larvae are subsequently ground mechanically to produce the insect powder.

Three forms of the NF are produced, i.e. whole, blanched and frozen larvae; whole, blanched and dried larvae; and powder of whole, blanched and dried larvae. The NF is stored in packaging certified for food contact (high density polyethylene mono material) at −18°C for the frozen form, and ambient temperature for the dried and powder forms. For all NF forms, the proposed shelf life is 6 months (further information on the NF's shelf life is provided in Section 3.4.1).

It has been previously discussed that *T. molitor* can be infected, e.g. by bacteria, parasites, entomopathogenic fungi and viruses, often as a result of poor hygiene farming conditions (EFSA NDA Panel, 2021a, 2021b; Precup et al.,2022). However, the Panel concludes that the production process steps implemented, and the specification limits set, may mitigate the risks of these biological hazards.

The Panel considers that the production process is sufficiently described.

3.4 | Compositional data

In order to confirm that the manufacturing process is consistent and adequate to produce on a commercial scale a product with certain characteristics, the applicant provided qualitative and quantitative data on chemical and microbiological parameters for a number of different batches of the NF forms (i.e. TM frozen; TM dried; TM powder). The Panel notes that not all the analyses have been performed on the same batches of the NF. Considering the production process, the Panel considers that the two forms of the NF (TM dried and TM powder) are representative of each other regarding most of their compositional parameters, excluding microbiological aspects and oxidative status of fats. The composition of TM dried and TM powder differs from TM frozen due to the reduced water content in the dried forms. Grinding increases the surface area of the NF, thus making TM powder more prone to deterioration.

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 not all of its constituents can be fully identified and/or characterised (EFSA NDA Panel, 2016).

TM frozen mainly consists of water, crude protein and carbohydrates, whereas the dried forms (TM dried, TM powder) mainly consist of crude protein, fat and carbohydrates. The results of the proximate analysis of the NF are presented in Table 1. The amino acid, fatty acid, vitamin and mineral compositions are reported in the Section 3.9.

[4]Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. OJ L 189, 20.7.2007, pp. 1–23.

[5]Commission Implementing Regulation (EU) 2021/1165 of 15 July 2021 authorising certain products and substances for use in organic production and establishing their lists. OJ L 253, 16.7.2021.

TABLE 1 Batch-to-batch proximate analysis of the NF forms.

	Batch number														
	TM frozen					TM dried					TM powder					
Parameter (unit)	#1	# 2	#3	# 4	#5	# 6	# 7	#8	#9	#10	#11	#12	#13	#14	#15	Analytical method
Crude protein (g/100 g of NF)	14.3	14.4	14.6	13.7	13.8	47.8	46.8	47.7	48.4	47.2	43.4	46.3	46.8	46.4	44.5	Kjeldahl (N x6.25)
Fat (g/100 g of NF)	6.0	6.0	6.1	5.6	5.9	34.4	21.4	34.5	31.2	35.3	22.6	19.4	19.2	19.4	20.2	Gravimetry (acid hydrolysis), Internal adaptation
Total carbohydrates (g/100 g of NF)	7.6	7.2	7.3	7.3	7.3	5.2	22.2	6.3	6.7	10.0	23.0	25.0	24.2	24.9	24.0	Calculation by difference (100-moisture, protein, fat, ash, fibre)
Dietary fibre[a] (g/100 g of NF)	1.8	1.9	1.0	1.8	1.5	4.4	3.5	3.0	5.4	1.7	4.9	3.6	4.8	4.0	2.8	Enzymatic-gravimetry (AOAC 991.43)
Sugars (g/100 g of NF)	< 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	IC-PAD, Internal adaptation
Ash (g/100 g of NF)	1.4	1.4	1.4	1.4	1.4	3.3	3.0	3.1	2.9	2.9	4.5	4.7	4.8	4.7	4.6	Gravimetry, Internal adaptation
Moisture (g/100 g of NF)	70.7	71.0	70.6	72.0	71.6	5.0	3.3	5.5	5.4	2.3	6.5	4.6	5.0	4.6	6.7	Thermo-gravimetry, Internal adaptation
Energy value (kcal/100 g of NF)	138	136	140	131	134	530	475	532	512	550	459	452	447	452	450	Regulation (EU) 1169/2011[b]

Abbreviations: AOAC, Association of Official Analytical Collaboration; IC–PAD, ion chromatography–pulsed amperometric detection; NF, novel food.

[a]The term is used as synonymous of non-digestible carbohydrates and does not reflect the additional requirement of having a beneficial physiological effect demonstrated by generally accepted scientific evidence laid down in Annex I of Regulation (EC) 1169/2011.

[b]Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. OJ L 304, 22.11.2011, pp. 18–63.

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

The Panel notes that the crude protein content of the NF listed in Table 1, which incorporates non-protein nitrogen into the calculation, overestimates the true protein content in the NF, primarily due to the presence of chitin (Janssen, Lakemond, et al., 2017). This point will be addressed in detail in Section 3.9.

Chitin is the main form of crude fibre[6] in *T. molitor* larvae (Finke, 2007; Hahn et al., 2018; Han & Heinonen, 2020). It is a linear polysaccharide consisting of varying amounts of β-(1,4)-linked 2-amino-2-deoxy-β-D-glucopyranose and 2-acetamido-2-deoxy-β-D-glucopyranose units (Muzzarelli & Raa, 1973; Roberts, 1992). The applicant provided analytical data on the levels of chitin in five independently produced batches of the NF for the dried and frozen forms and in three batches for the powder form (Table 2). The Panel notes that a nationally or internationally recognised reference method for the analytical determination of chitin levels does not exist. The chitin content in the NF was determined using two methods, the acetate-based measurement method (acetyl method) and another one based on the protocol described by Hahn et al. (2018), in which chemical treatment [based on acid detergent fibre (ADF)–acid detergent lignin (ADL)] is used to estimate the chitin content (Table 2). Three additional independently produced batches on the chitin content were provided for the TM powder and two batches for the TM frozen, with an average (± SD) content of 3.6 g/100 g (± 0.1) (TM powder) and 3.5 g/100 g (± 0.4) (TM frozen), determined by acetyl group determination method. The Panel considers that the differences between the content of dietary fibre (Table 1) and chitin (Table 2) could be due to the different analytical methods utilised. Additionally, the Panel notes that the analytical results in Tables 1 and 2 do not concern the same NF batches.

TABLE 2 Chitin content in the NF, on a product basis.

	Batch number													
	TM frozen					TM dried					TM powder			
Parameters (unit)	#18	#19	#3	#21	#22	#23	#24	#25	#26	#27	#28	#29	#30	Analytical method
ADF (g/100 g)	7.9	8.0	2.7	2.6	3.6	7.8	7.2	7.1	7.1	6.9	8.2	8.6	10.8	AFNOR method NF V18-122
ADL (g/100 g)	1.4	4.9	0.4	0.6	0.7	1.4	1.2	1.3	1.1	1.1	1.5	1.5	2.6	AFNOR method NF V18-122
Chitin[a] (g/100 g)	6.5	3.1	2.3	2	2.9	6.4	6.0	5.8	6.0	5.8	6.7	7.1	8.2	Calculation (ADF-ADL)

Abbreviations: ADF, acid detergent fibre; ADL, acid detergent lignin.

[a]Chitin calculated as ADF–ADL, using the Afnor standard NF V18-122 (Association Française de Normalisation (AFNOR)), method by treatment with neutral and acid detergent and sulfuric acid.

The content of heavy metals in the NF determined by inductively coupled plasma-mass spectrometry (ICP-MS) is reported in Table 3. Upon EFSA's request, noting the cadmium content of batches #31, #32, #33 of TM frozen and lead content of batch #42 of TM dried, the applicant analysed additional batches (#34, #35, #36, #37, #48, #49, #50, #51), obtaining results in compliance with the respective proposed specification limit. The applicant compared the analytical values to the maximum levels (MLs) for other foods as set in Regulation (EU) 2023/915.[7] The Panel notes that the content of heavy metals reported for the NF are below maximum levels set for other foods, and that they are similar to the content previously reported and assessed for other foods derived from whole insects (EFSA NDA Panel, 2021a, 2021b, 2021c, 2021d), and that in the current EU legislation, no maximum levels of heavy metals are set for insects and products thereof as food.

[6]The term 'dietary fibre' in the context of this opinion is used as defined by the EFSA NDA Panel (2010), i.e. all non-digestible carbohydrates, and not as defined by Regulation (EU) 1169/2011, which sets the additional requirement of having a beneficial physiological effect for edible carbohydrate polymers obtained from food raw material by physical, enzymatic or chemical means, and for edible synthetic carbohydrate polymers.

[7]Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006 OJ L 119, 5.5.2023, pp. 103–157.

TABLE 3 Heavy metals in the NF.

Heavy metals (mg/kg)	Batch number																						Analytical method
	TM frozen									TM dried					TM powder								
	#18	#19	#31	#32	#33	#34	#35	#36	#37	#10	#38	#39	#40	#41	#42	#43	#44	#45	#46	#47	#48	#49	#50	#51	
Lead	< 0.01	< 0.01	/	< 0.01	< 0.01	/	/	/	/	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.061	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	Internal method (digestion according NEN-EN 13805), ICP-MS
Cadmium	0.03	0.03	0.061	0.61	0.057	0.035	0.036	0.037	0.035	0.1	0.10	0.1	0.1	0.093	/	0.098	0.10	0.1	0.089	0.098	/	/	/	/	
Mercury	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	/	/	/	/	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.030	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	/	/	/	/	
Arsenic	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	/	/	/	/	< 0.02	< 0.02	< 0.02	0.021	< 0.02	< 0.01	0.022	0.029	< 0.02	< 0.02	0.022	/	/	/	/	

Note: /: not provided.

Abbreviation: ICP-MS, inductively coupled plasma-mass spectrometry.

Analytical data on the levels of aflatoxins[8] B1, B2, G1, G2, ochratoxin A,[9] deoxynivalenol,[10] fumonisins B1 and B2,[11] and zearalenone[12] in the NF have been provided. The values reported are below the LOQ of the analytical methods implemented [Internal method, liquid chromatography–tandem mass spectrometry (LC–MS/MS)]. The LOQ values are lower than the MLs set for different foods in Regulation (EU) 2023/915. The Panel notes that in the current EU legislation, no MLs of mycotoxins are set for insects as food.

Additionally, the concentrations of perfluoroalkyl substances (PFAS), polycyclic aromatic hydrocarbons (PAHs), dioxins and PCBs in the NF were provided by the applicant (Table 4, Appendix C) and the values reported were lower than the MLs set for different foods in Regulation (EU) 2023/915, and comparable to those previously reported and assessed for other foods derived from whole insects (EFSA NDA Panel, 2021a, 2021b, 2021c, 2021d). The Panel notes that in the current EU legislation, no MLs of dioxins and dioxin-like compounds, PFAS or PAHs are set for insects as food.

Regarding processing contaminants, the applicant provided data on acrylamide and chloropropanols (2- and 3-MCPD) for the powder form, with values < LOQ (< 5 μg/kg) (Table 4). The Panel considers that the concentrations of the analysed processing contaminants do not raise safety concerns.

TABLE 4 Processing contaminants and halogenated persistent organic pollutants in TM powder.

	Batch number					
Parameters (μg/kg)	**#11**	**#12**	**#13**	**#14**	**#15**	**Analytical method**
PFAS (μg/kg)						
Perfluorooctane sulphonic acid (PFOS)	< 0.100	< 0.100	< 0.100	< 0.100	< 0.100	Internal method, LC-MS/MS
Perfluorooctanoic acid (PFOA)	< 0.100	< 0.100	< 0.100	< 0.100	< 0.100	
Perfluorononanoic acid (PFNA)	< 0.100	< 0.100	< 0.100	< 0.100	< 0.100	
Perfluorhexanesulfonic acid (PFHxS)	< 0.100	< 0.100	< 0.100	< 0.100	< 0.100	
PAHs (μg/kg)						
Benz(a)anthracene	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	
Benzo(a)pyrene	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	
Benzo(b)fluoranthene	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	
Chrysene	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	
Dioxins (pg/g fat)						
WHO(2005)[a]-PCDD/F+dl-PCB TEQ (upper-bound)	4.28	3.62	4.22	3.12	4.52	Conform EC 2017/644 (food) and EC 2017/771 (feed)
PCDD/F TEQ (upper-bound)	1.30	0.855	1.12	0.822	0.929	
ndl-PCBs (ng/g fat)						
PCB 28	0.831	0.958	1.02	1.05	0.992	
PCB 52	1.66	1.45	1.68	1.59	1.73	
PCB 101	9.61	7.70	9.91	10.8	10.9	
PCB 138	13.4	10.3	13.8	14.3	15.2	
PCB 153	19.5	14.3	24.5	23.2	21.4	
PCB 180	15.9	12.5	17.1	18.6	18.6	
Furan (μg/kg)	< 5	< 5	< 5	< 5	< 5	US FDA/CFSAN 2006-10; mod.
Acrylamide (μg/kg)	< 30	< 30	< 30	< 30	< 30	Internal Method, LC–MS/MS
2-Monochloropropanediol (free-form) (μg/kg)	< 5	< 5	< 5	< 5	< 5	Internal Method, GC-MS/MS
3-Monochloropropanediol (free-form) (μg/kg)	< 5	< 5	< 5	< 5	< 5	Internal Method, GC–MS/MS

Abbreviations: GC–MS/MS, gas chromatography–tandem mass spectrometry; LC–MS/MS, liquid Chromatography–tandem mass spectrometry; ndl- PCBs, non dioxin-like polychlorinated biphenyls; PAHs, polycyclic aromatic hydrocarbons; PCDD/F, polychlorinated dibenzo-para-dioxins and polychlorinated dibenzofurans; PFAS, perfluoroalkyl substances; US FDA/CFSAN, United States Food and Drug Administration/The Center for Food Safety and Applied Nutrition.

[a]Van den Berg et al. (2006).

Analytical data on pesticide residues in five independently produced batches of all forms of the NF have been provided. The results showed that the concentrations of tested pesticides residues in the NF are below the limits of detection (LOD) or quantification (LOQ) (0.01 mg/kg) of the analytical multimethod used [gas chromatography with tandem mass spectrometry (GC–MS/MS); LC–MS/MS].

[8]B1; B2; G1; G2: < 0.1 μg/kg (LOQ) for all forms.

[9]Ochratoxin A: < 0.2 μg/kg (LOQ) for all forms.

[10]Deoxynivalenol: < 20 μg/kg (LOQ) for all forms.

[11]Fumonisins B1 and B2: < 20 μg/kg (LOQ) for all forms.

[12]Zearalenone: < 10 μg/kg (LOQ) for all forms.

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

The applicant provided microbiological data on five independently produced batches of the NF (Tables 5–7).

TABLE 5 Microbiological analyses of the TM frozen.

	Batch number					
	TM frozen					
Parameter (unit)	**#18**	**#19**	**#42**	**#43**	**#44**	**Analytical method**
Aerobic plate count (30°C) (CFU/g)	170	90	70	150	270	ISO 4833-1
Yeasts and moulds (CFU/g)	< 100	< 100	< 100	< 100	< 100	ISO 21527-2
Sulfite-reducing anaerobes (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 15213 at 37°C
Presumptive *Bacillus cereus* (CFU/g)	< 100	< 100	< 100	< 100	< 100	ISO 7932
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10	< 10	< 10	3M 01/06–09/97
β-Glucuronidase-positive *E. coli* (CFU/g)	< 10	< 10	< 10	< 10	< 10	BRD 07/01–07/93
Salmonella in 25 g	N.D.	N.D.	N.D.	N.D.	N.D.	AFNOR EGS 38/01–03/15
Coagulase positive staphylococci (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 6888.2
Listeria monocytogenes in 25 g	N.D.	N.D.	N.D.	N.D.	N.D.	BRD 07/04–09/98
Clostridium perfringens (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 7937

Abbreviations: CFU, colony forming unit; N.D., not detected.

TABLE 6 Microbiological analyses of the TM dried.

	Batch number					
	TM dried (whole)					
Parameter (unit)	**#6**	**#45**	**#46**	**#47**	**#10**	**Analytical method**
Aerobic plate count (30°C) (CFU/g)	1.1×10^3	2.2×10^3	350	4.2×10^3	5.4×10^3	ISO 4833-1
Yeasts and moulds (CFU/g)	< 100	< 100	< 100	< 100	< 100	ISO 21527-2
Sulfite-reducing anaerobes (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 15213
Presumptive *Bacillus cereus* (CFU/g)	< 100	< 100	< 100	< 100	< 100	ISO 7932
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10	< 10	< 10	3M 01/06–09/97
β-Glucuronidase-positive *E. coli* (CFU/g)	< 10	< 10	< 10	< 10	< 10	BRD 07/01–07/93
Salmonella in 25 g	N.D.	N.D.	N.D.	N.D.	N.D.	AFNOR EGS 38/01–03/15
Listeria monocytogenes in 25 g	N.D.	N.D.	N.D.	N.D.	N.D.	BRD 07/04–09/98
Coagulase positive staphylococci (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 6888-2
Clostridium perfringens (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 7937

Abbreviations: CFU, colony forming unit; N.D., not detected.

TABLE 7 Microbiological analyses of the TM powder.

	Batch number										
	TM powder										
Parameter (unit)	**#48**	**#49**	**#50**	**#51**	**#52**	**#53**	**#54**	**#55**	**#56**	**#57**	**Analytical method**
Aerobic plate count (30°C) (CFU/g)	1.1×10^5	1.3×10^5	1.1×10^5	3.2×10^4	10^5	590	2.5×10^3	390	250	660	ISO 4833-1
Yeasts (CFU/g)	< 10	< 10	< 10	< 10	< 10	/	/	/	/	/	ISO 21527-2
Moulds (CFU/g)	< 10	20	< 10	10	10	/	/	/	/	/	ISO 21527-2
Sulfite-reducing anaerobes (CFU/g)	< 10	< 10	15	< 10	5	/	/	/	/	/	ISO 15213 at 37°C

TABLE 7 (Continued)

	Batch number										
	TM powder										
Parameter (unit)	**#48**	**#49**	**#50**	**#51**	**#52**	**#53**	**#54**	**#55**	**#56**	**#57**	**Analytical method**
Presumptive *Bacillus cereus* (CFU/g)	< 100	< 100	< 100	< 100	< 100	/	/	/	/	/	ISO 7932 + spiral
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10	< 10	100	/	/	/	/	/	NF V08-054
β-Glucuronidase-positive *E. coli* (CFU/g)	< 10	< 10	< 10	< 10	< 10	/	/	/	/	/	ISO 16649-2 + spiral
Salmonella in 25 g	N.D.	N.D.	N.D.	N.D.	N.D.	/	/	/	/	/	RAPID/Salmonella
Coagulase positive staphylococci (CFU/g)	< 10	< 10	< 10	< 10	< 10	/	/	/	/	/	ISO 6888-2
Listeria monocytogenes in 25 g	N.D.	N.D.	N.D.	N.D.	N.D.	/	/	/	/	/	RAPID'L. MONO
Clostridium perfringens (CFU/g)	< 10	< 10	< 10	< 10	< 10	/	/	/	/	/	ISO 7937

Abbreviations: CFU, colony forming unit; N.D., not detected.

The Panel noted the variations on aerobic plate count and upon EFSA's request, the applicant provided additional analytical data on five independently produced batches, which were aligned with the specification limit for this parameter.

Regarding biogenic amines, the applicant provided a literature review upon EFSA's request. Analytical data were provided on histamine levels in five independently produced batches of the TM powder. The histamine levels (< 1 mg/kg) were lower than the limit of 200 mg/kg for histamine in fishery products set in Commission Regulation (EC) No 2073/2005.[13]

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

3.4.1 | Stability

The applicant provided data on the microbiological profile of five batches of TM frozen and TM dried, and upon EFSA's request on TM powder. The NF forms have been analysed immediately after manufacturing (0 months) and after storage at room temperature (TM dried and TM powder) or −18°C (TM frozen) for 7 months. The Panel notes that the five batches of the NF analysed at $t = 7$ months are not the same five NF batches analysed at $t = 0$ months. The Panel also notes that the microbiological values do not exceed the given specification limits (Tables 8–10).

TABLE 8 Microbiological status of the TM frozen during the proposed shelf life.

Parameter	**Batch number**										
Time (months)	**0**					**7**					
TM frozen	**#58**	**#59**	**#60**	**#61**	**#62**	**#63**	**#64**	**#65**	**#66**	**#67**	**Analytical method**
Aerobic plate count (30°C) (CFU/g)	50	1.1×10^3	100	10^3	50	170	90	70	150	270	ISO 4833-1
Yeasts and moulds	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	ISO 21527-2
Sulpfite-reducing anaerobes (CFU/g)	< 10	< 100	< 100	< 10	< 10	< 10	< 10	< 100	< 10	< 100	ISO 15213
Clostridium perfringens (CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	ISO 7937
Presumptive *Bacillus cereus* (CFU/g)	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	ISO 7932

(Continued)

[13]Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338,22.12.2005, pp. 1–26.

TABLE 8 (Continued)

Parameter	Batch number										
Time (months)	0					7					Analytical method
TM frozen	#58	#59	#60	#61	#62	#63	#64	#65	#66	#67	
L. monocytogenes in 25 g	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	BRD 07/04–09/98
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10	< 40	< 10	< 10	< 10	< 10	< 10	< 10	3M 01/06–09/97
β-Glucuronidase-positive *Escherichia coli* (CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	BRD 07/01–07/93
Salmonella in 25 g	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	AFNOR EGS 38/01–03/15
Coagulase positive staphylococci (CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	ISO 6888-2

Abbreviations: a_w, water activity; CFU, colony forming unit; N.D., not detected.

TABLE 9 Microbiological status of the TM dried during the proposed shelf life.

Parameter	Batch number										
Time (months)	0					7					Analytical method
TM dried	#68	#69	#70	#71	#72	#68	#69	#70	#71	#72	
Aerobic plate count (30°C) (CFU/g)	1.2×10^3	9.8×10^2	/	30×10^3	30×10^3	1.1×10^3	2.2×10^3	3.5×10^1	4.2×10^3	5.4×10^3	ISO 4833-1
Yeasts	400	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	ISO 21527-2
Moulds	< 100	< 400	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	ISO 21527-2
Sulfite-reducing anaerobes (CFU/g)	< 10	< 100	< 10	< 10	< 10	< 10	< 10	< 100	< 10	< 100	ISO 15213
Clostridium perfringens (CFU/g)	< 10	< 10	/	/	/	< 10	< 10	< 10	< 10	< 10	ISO 7937
Presumptive *Bacillus cereus* (CFU/g)	< 100	< 100	/	< 100	< 100	< 100	< 100	< 100	< 100	< 100	ISO 7932
L. monocytogenes in 25 g	N.D.	N.D.	/	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	BRD 07/04–09/98
Enterobacteriaceae (CFU/g)	< 10	< 10	/	/	/	< 10	< 40	< 10	< 10	< 10	3M 01/06–09/97
β-Glucuronidase-positive *Escherichia coli* (CFU/g)	< 10	< 10	/	< 10	< 10	< 10	< 10	< 10	< 10	< 10	BRD 07/01–07/93
Salmonella in 25 g	N.D.	N.D.	/	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	AFNOR EGS 38/01–03/15
Coagulase positive staphylococci (CFU/g)	< 10	< 10	/	< 10	< 10	< 10	< 10	< 10	< 10	< 10	ISO 6888-2
a_w	/	/	/	0.287	0.358	/	0.352[a]	0.360[a]	0.287[a]	0.358[a]	Internal, Hygrometry (dew-point)

Abbreviations: a_w, water activity; CFU, colony forming unit; N.D., not detected.

[a]The analytical data refer to different batches than those analysed for microbiological parameters.

TABLE 10 Microbiological status of the TM powder during the proposed shelf life.

Parameter	**Batch number**										
Time (months)	**0**					**7**					
Batch number	**#73**	**#74**	**#75**	**#76**	**#77**	**#78**	**#79**	**#80**	**#81**	**#82**	**Analytical method**
Aerobic plate count (30°C) (CFU/g)	1.1×10^5	1.3×10^5	1.1×10^5	3.2×10^4	10^5	6.8×10^3	2.4×10^3	8×10^3	4×10^3	6.8×10^3	PCA Agar-P ISO 4833-1
Yeasts (CFU/g)	< 10	< 10	< 10	< 10	< 10	< 100	< 100	< 100	< 100	< 400	ISO 21527-2
Moulds (CFU/g)	< 10	20	< 10	10	10	< 100	< 100	< 100	< 100	< 100	ISO 21527-2
Sulfite-reducing anaerobes (CFU/g)	< 10	< 10	15	< 10	5	< 10	< 10	< 10	< 10	< 10	ISO 15213
Clostridium perfringens (CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	ISO 7937
Presumptive *Bacillus cereus* (CFU/g)	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	ISO 7932
L. monocytogenes in 25 g	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	BRD 07/04–09/98
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10	< 10	100	< 10	< 10	< 10	< 10	< 10	3M 01/06–09/97
β-Glucuronidase-positive *Escherichia coli* (CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	BRD 07/01–07/93
Salmonella in 25 g	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	AFNOR EGS 38/01–03/15
Coagulase positive staphylococci (CFU/g)	< 100	< 100	< 100	< 100	< 100	< 100	< 10	< 100	< 100	< 100	ISO 6888-2

Abbreviations: CFU, colony forming unit; N.D., not detected.

Following EFSA's request, the applicant provided analytical data on the water activity (TM powder) and the oxidative status of the fat in the NF (TM frozen and TM powder) (Table 11).

The Panel notes that the samples analysed at $t=0$ were not always the same NF batches analysed at later time points. Nevertheless, the Panel notes that the values of none of the analysed bathes exceeded the given specification limits.

The peroxide values (PV), *p*-anisidine values and free-fatty acids (FFA) percentages were determined for a period ($t=7$ months), that covers the proposed shelf life (6 months). The Panel noted the high variability among the p-anisidine and FFA values. The applicant indicated that the five batches of the NF analysed at $t=7$ months are not the same five NF batches analysed at $t=0$ months. Thus, the Panel notes that no monitoring results of specific NF batches over time were provided.

TABLE 11 Water activity and oxidative status of fat in the TM (frozen and powder) during the proposed shelf life.

Parameter	**Batch number**										
Time (months)	**0 months**					**7 months**					**Analytical method**
TM frozen											
	#83	**#84**	**#85**	**#86**	**#87**	**#88**	**#89**	**#90**	**#91**	**#92**	
FFA (% in fat or extracted oil)	0.99	1.41	0.81	1.03	1.15	12.63	6.36	6.46	6.05	8.74	ISO 660:2009
PV (meq O_2/kg fat)	1.0	0.9	1.0	1.5	/	< 0.4	< 0.4	0.7	0.7	0.7	ISO 27107:2009
	#18	**#19**	**#42**	**#43**	**#44**	**#63**	**#64**	**#65**	**#66**	**#67**	
p-Anisidine value	2.76	1.39	2.24	0.77	5.27	1.06	0.62	0.75	0.84	0.91	AOCS Cd 18–90 (2017)
TM powder											
	#93	**#94**	**#95**	**#96**	**#97**	**#78**	**#79**	**#80**	**#81**	**#82**	
a_w	0.418	0.429	0.437	0.426	0.420	/	/	/	/	/	Internal, Hygrometry (dew-point)
FFA (% in fat or extracted oil)	0.90	1.02	1.14	0.88	0.90	7.71	7.71	9.19	8.09	5.46	ISO 660:2009
PV (meq O_2/kg fat)	1.4	1.3	1.3	1.6	1.4	1.9	1.1	1.9	1.9	2.3	ISO 27107:2009
	#11	**#12**	**#13**	**#14**	**#15**	**#98**	**#99**	**#100**	**#101**	**#102**	
p-Anisidine value (LOQ)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	1.26	1.58	4.36	40.21	AOCS Cd 18–90 (2017)

Abbreviations: a_w, water activity; FFA, free-fatty acids; meq, milliequivalents; PV, peroxide value.

Histamine levels were quantified at the end of the proposed shelf life in the TM powder and were below the LOQ (< 1 mg/kg). The stability data on microbial contamination or lipid hydrolysis did not raise safety concern. Provided that the specifications of the NF are met also at the end of the shelf life the stability data do not raise safety concerns. The Panel considers that the data provided sufficient information with respect to the stability of the NF with a shelf life of 6 months.

3.4.2 | Stability in the intended-for-use food matrices

Since the NF is going to be used as an ingredient of 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).

The applicant investigated the formation of processing contaminants, i.e. acrylamide (LC–MS/MS), furan in pasta and biscuits prepared with the NF as an ingredient (two samples per food category) and in mealworm paste. The pasta was prepared by adding the NF to wheat semolina and eggs at a use level of 10% and the dough was shaped and dried for 12 to 15 h at 45°C and stored for 5 months at ambient conditions. For obtaining the biscuits, the NF was added to the other ingredients at a use level of 5% and baked for 12 min at 180°C in a hot air oven and stored for 1 month at ambient conditions. Appropriate control samples (pasta and biscuits produced in the same way but without the NF) were provided. Mealworm paste was obtained by using 81% blanched NF larvae, that were further pulverised, mixed with spices and binding agents and finally stir-fried (30 min at 100°C) to obtain a product similar to minced meat, which was stored at –18°C for 18 months. A sample for comparison was not provided.

The concentration of furan in the pasta containing the NF and in the control product was < 5 μg/kg, acrylamide < 30 μg/kg. In biscuits with the NF, furan was 8.3 μg/kg compared to < 5 μg/kg in the control and acrylamide levels were higher in the biscuits with the NF (250 μg/kg) compared to 110 μg/kg in the control, but below the benchmark level for biscuits. Levels of furan and acrylamide in the mealworm paste were reported as < 5 and 52 μg/kg, respectively.

The applicant provided data on the microbiological profile and oxidative stability (peroxide value, free-fatty acids) of three intended-for-use matrices (pasta, biscuits and mealworm paste) with the NF as an ingredient analysed at the end of shelf life (Tables 12 and 13).

TABLE 12 Microbiological stability in the intended-for-use matrices at the end of shelf life.

	Intended-for-use matrices						
	Pasta with TM		Control	Biscuits with TM	Control	Mealworm paste	
Parameter	#103	#104	#105	#106	#107	#108	Analytical method
Aerobic plate count (30°C) (CFU/g)	< 10	< 10	< 10	340	70	2300	ISO 4833-1
Heat-resistant mesophilic spore count	< 10	< 10	< 10	< 10	< 10	120	Internal Method, E-Cultural technique (non-chromogenic media)
Presumptive *Bacillus cereus* (CFU/g)	< 100	< 100	< 100	< 100	< 100	400	ISO 7932
Clostridium perfringens (CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10	ISO 7937

Abbreviation: CFU, colony forming unit.

TABLE 13 Processing contaminants and oxidative stability in the intended-for-use matrices at the end of shelf life.

	Pasta with NF		Control	Biscuits with NF		Control		Minced meat with NF		
Parameter	#103	#104	#105	#106	#107	#108	#109	#110	#111	Analytical method
Furan (µg/kg)	< 5	< 5	< 5	8.3	9.3	< 5	< 5	< 5	/	US FDA/CFSAN 2006–2010; mod.
Acrylamide (µg/kg)	< 30	< 30	< 30	250	180	45	110	52	51	Internal Method, LC–MS/MS
Histamine (mg/kg)	< LOQ	/	< LOQ	< LOQ	/	/	< LOQ	< LOQ	/	Czech J. Food Sci. Vol.21
PV (meq O_2/kg fat)	/	/	/	< 0.4	/	/	< 0.4	0.5	/	ISO 27107:2009
FFA (% in fat or extracted oil)	/	/	/	1.10	/	/	1.22	1.37	/	ISO 660:2009

Note: /, data not provided (due to too little fat extraction).

The Panel notes that the analytical data regarding the putative formation of contaminants, lipid oxidation and microbiological content on the use of NF as an ingredient in pasta, biscuits and in mealworm paste are limited. The Panel further notes that the food items containing the NF have to comply with currently established legislative limits, such as microbiological levels set in Regulation (EC) No 2073/2005 and the benchmark levels of acrylamide in bakery products established by Regulation (EU) No 2017/2158.[14]

The stability data on microbial contamination or lipolysis and lipid 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 contaminants, the stability data do not raise safety concerns.

3.5 | Specifications

The specifications of the NF are indicated in Table 14.

[14]Commission regulation (EU) 2017/2158 of 20 November 2017 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. OJ L 304, 21.11.2017, pp. 24–44. Safety of frozen and dried formulations from whole yellow mealworm (*Tenebrio molitor* larva) www.efsa.europa.eu/efsajournal 13 *EFSA Journal* 2021;19(8):6778.

TABLE 14 Specifications of the NF.

Description			
TM frozen: Whole, blanched and frozen *T. molitor* larva			
TM dried: Whole, blanched and dried *T. molitor* larva			
TM powder: Whole, blanched, dried and ground *T. molitor* larva (powder)			
Parameter	**Unit**	**TM frozen**	**TM dried or TM powder**
Appearance		Yellow-brown colour, 1–2.5 cm length with ringed, segmented bodies	Yellow-brown colour
Moisture	% w/w	70–75	2–8
Crude protein (*N* × 6.25)	% w/w	13–20	56–64
Fat	% w/w	5–9	19–35
Of which saturated	% fat	0.9–1.9	4–9.0
Digestible carbohydrates	% w/w	3.5–8.0	5.0–27.0
Dietary fibre	% w/w	0.8–2.6	1.5–7.0
Chitin*	% w/w	2.0–6.5	3.5–8.2
Water activity	–	< 1	< 0.6
Peroxide value	meq O_2/kg fat	< 2	≤ 5
***p*-Anisidine value**	–	< 1.1	< 5
Ash	% w/w	< 2.5	< 6.0
Minerals			
Manganese	mg/kg	≤ 5	≤ 11
Heavy metals			
Lead	mg/kg	≤ 0.01	≤ 0.01
Cadmium	mg/kg	≤ 0.05	≤ 0.1
Mercury	mg/kg	≤ 0.01	≤ 0.01
Arsenic	mg/kg	≤ 0.02	< 0.06
Mycotoxins			
Aflatoxin B1	µg/kg	< 0.1	
Aflatoxin B2	µg/kg	< 0.1	
Aflatoxin G1	µg/kg	< 0.1	
Aflatoxin G2	µg/kg	< 0.1	
Aflatoxins (sum of B1 + B2, G1 + G2)	µg/kg	< 0.4	
Fumonisin B1 + B2	µg/kg	< 50	
Ochratoxin A	µg/kg	< 0.5	
Deoxynivalenol	µg/kg	< 25	
Zearalenone	µg/kg	< 15	
Microbiological parameters			
Aerobic mesophilic bacteria	CFU/g	≤ 10^5	
Enterobacteriaceae (presumptive)	CFU/g	≤ 100	
Beta-Glucuronidase positive *Escherichia coli*	CFU/g	< 10	
Listeria monocytogenes	in 25 g	Not detected	
Salmonella spp.	in 25 g	Not detected	
Bacillus cereus (presumptive)	CFU/g	< 100	
Clostridium perfringens	CFU/g	≤ 10	
Coagulase positive staphylococci	CFU/g	≤ 100	
Sulfite-reducing anaerobes	CFU/g	≤ 10	
Yeasts and moulds	CFU/g	≤ 100	
Other contaminants			
Histamine	mg/kg	< 5	< 5
Perfluoroalkyl substances	µg/kg	< 0.1	
Sum of all positive identified PAHs	µg/kg	< 10	

TABLE 14 (Continued)

Description			
TM frozen: Whole, blanched and frozen *T. molitor* larva			
TM dried: Whole, blanched and dried *T. molitor* larva			
TM powder: Whole, blanched, dried and ground *T. molitor* larva (powder)			
Parameter	**Unit**	**TM frozen**	**TM dried or TM powder**
Sum of dioxins and dioxin-like PCBs (WHO-PCDD/F- PCB-TEQ)	pg/g fat	< 5	

Abbreviations: PAHs, polycyclic aromatic hydrocarbons; sum of polychlorinated dibenzo-p-dioxins-polychlorinated dibenzofurans-polychlorinated biphenyls; WHO, World Health Organization.

*Chitin calculated as the difference between the acid detergent fibre fraction and the acid detergent lignin fraction (ADF-ADL), as described by Hahn et al. (2018).

The Panel considers that the specification limit of 10 meq O_2/kg fat for peroxide value in the dried forms is too high and considers a limit of 5 meq O_2/kg fat to be appropriate for the novel food in the dried and powder forms.

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

The source of the NF is the yellow mealworm (*T. molitor* larva). Yellow mealworms are consumed as part of the customary diet or for medicinal purposes in some non-EU countries (Thailand, China and Mexico) (Feng et al., 2018; Hanboonsong et al., 2013; Ramos-Elorduy, 1997, 2009; Ramos-Elorduy & Moreno, 2004). Since 1 June 2021, certain food products from the same source (yellow mealworm) are authorised in the EU market for human consumption [Commission Implementing Regulation (EU) 2021/88].[15,16]

3.7 | Proposed uses and use levels and anticipated intake

3.7.1 | Target population

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

3.7.2 | Proposed uses and use levels

The NF is proposed to be used as ingredient in several food products. The food categories defined using the FoodEx2 hierarchy (EFSA, 2015) and the maximum use levels are reported in Table 15.

TABLE 15 Food categories and maximum use levels intended by the applicant.

FoodEx2level	FoodEx2 code	Food category	Max use level TM frozen (g NF/100 g)	Max use level TM dried and TM powder (g NF/100 g)
3	A005Y	Crackers and breadsticks	21	9
3	A03NS	Liqueurs	2	1
3	A03PD	Unsweetened spirits	2	1
3	A06HL	Snacks other than chips and similar	100	100
3	A04MN	Fruit/vegetable spreads and similar	10	4
4	A005R	Gluten free bread	15	6
4	A009X	Biscuits, sweet, plain	15	6

(Continued)

[15]Commission Implementing Regulation (EU) 2021/882 of 1 June 2021 authorising the placing on the market of dried Tenebrio molitor larva as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council and amending Commission Implementing Regulation (EU) 2017/2470. OJ L 194/16, 2.6.2021.

[16]Commission Implementing Regulation (EU) 2022/169 of 8 February 2022 authorising the placing on the market of frozen, dried and powder forms of yellow mealworm (*Tenebrio molitor* larva) as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council, and amending Commission Implementing Regulation (EU) 2017/2470. OJL 28, 9.2.2022.

TABLE 15 (Continued)

FoodEx2level	FoodEx2 code	Food category	Max use level TM frozen (g NF/100 g)	Max use level TM dried and TM powder (g NF/100 g)
4	A00AE	Biscuit with inclusions, filling or coating	15	6
4	A00EZ	Cereal bars plain	23	10
4	A00FA	Cereal bars mixed	23	10
4	A03YV	Mushroom salad	21	9
4	A040C	Finger food	83	32
4	A041M	Onion soup	21	9
4	A041N	Tomato soup	21	9
4	A041Q	Legume (beans) soup	21	9
4	A041R	Mushroom soup	21	9
4	A042E	Caesar salad	21	9
4	A042F	Greek salad	21	9
4	A0B9R	Mixed vegetables soup, dry	21	9
4	A0B9S	Mushroom soup, dry	21	9
4	A0B9X	Tomato soup, dry	21	9
4	A042G	Prepared legume (beans) salad	21	9
4	A042H	Prepared pasta salad	21	9
4	A042J	Prepared rice salad	21	9
4	A042K	Prepared nut salad	21	9
4	A042M	Prepared mixed egg/meat/fish/vegetable salad	21	9
4	A0CDN	Mixed soups	21	9
4	A0CEN	Savoury pies and tarts	21	9
5	A007F	Fresh pasta	23	10
5	A007T	Fresh stuffed pasta	23	10
5	A007L	Dried pasta	23	10
5	A007Y	Dried stuffed pasta	23	10
5	A008B	Pasta, gluten free	23	10
5	A008C	Couscous	23	10
5	A008D	Gnocchi	23	10
5	A008E	Glass noodle	23	10
5	A008F	Noodle, rice	23	10
5	A041C	Nasi goreng	23	10
5	A041D	Paella	23	10
5	A041F	Risotto	23	10
5	A041G	Rice and vegetables meal	21	9
5	A041J	Rice, meat and vegetables meal	21	9
5	A04LC	Pasta wholemeal	23	10
5	A0CDP	Pasta, filled, cooked	23	10
5	A0CDQ	Pasta, plain (not stuffed), cooked	23	10
5	A034G	Bitter chocolate	15	6
5	A034S	Pralines	15	6
5	A0C6P	Chocolate spread	15	6
5	A034R	Chocolate coated confectionery	15	6

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 16), using the EFSA Dietary Exposure (DietEx) Tool,[17] which is based on individual data

[17] https://www.efsa.europa.eu/it/science/tools-and-resources/dietex.

from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011). Since the applicant intends to use the different NF forms individually and not in combination in the respective food categories, the scenario that leads to the inclusion of the highest amount of NF dry matter in each food category was used to calculate anticipated daily intakes. The lowest and highest means and 95th percentiles of the anticipated daily intake of the NF (on a mg/kg body weight (bw) basis and as mg per day), among the EU dietary surveys, are presented in Tables 16 and 17.

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 16 Intake estimate (mg/kg bw per day) of the NF resulting from its use as an ingredient in all intended food categories at the maximum proposed use levels.

		Mean intake (mg/kg bw per day)		P95 intake (mg/kg bw per day)	
Population group	**Age (years)**	**Lowest[a]**	**Highest[a]**	**Lowest[b]**	**Highest[b]**
Infants	< 1	9.7	110.6	50.3	745.0
Young children[d]	1 to < 3	24.7	344.8	78.0	883.3
Other children	3 to < 10	9.6	346.4	48.6	904.0
Adolescents	10 to < 18	4.3	162.9	22.3	428.0
Adults[c]	≥ 18	9.4	203.5	26.9	552.8

Abbreviations: bw, body weight; P95, 95th percentile.

[a]Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 29/10/2024. 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 29/10/2024. The lowest and the highest P95 observed among all EU surveys are reported in these columns (P95 based on less than 60 individuals are not considered).

[c]Includes elderly, very elderly, pregnant and lactating women.

[d]Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).

TABLE 17 Intake estimate (mg per day) of the NF resulting from its use as an ingredient in the intended food categories at the maximum proposed use levels.

		Mean intake (mg per day)		P95 intake (mg per day)	
Population group	**Age (years)**	**Lowest[a]**	**Highest[a]**	**Lowest[b]**	**Highest[b]**
Infants	< 1	90.7	969.5	445.7	7800.0
Young children[c]	1 to < 3	250.1	4401.4	986.4	10600.0
Other children	3 to < 10	177.2	7488.0	798.1	19500.0
Adolescents	10 to < 18	226.7	8043.4	1377.6	20511.2
Adults[d]	≥ 18	687.5	13878.1	1874.0	35842.0

[a]Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 29/10/2024. 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 29/10/2024. The lowest and the highest P95 observed among all EU surveys are reported in these columns (P95 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.

3.7.4 | Estimate of exposure to undesirable substances

Based on the highest P95 intake estimates (Tables 16 and 17), EFSA calculated the exposure to undesirable substances (heavy metals, mycotoxins and organic contaminants) from the NF, for all population groups. The specification limits (Table 13) were used as maximum values for the concentrations of substances considered. 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 intake of undesirable substances through diet. The assessment of the intake of manganese (Mn) from the NF is provided in Section 3.9.

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

No ADME data have been provided for the NF.

3.9 | Nutritional information

The applicant provided nutritional analysis of the NF. The frozen form consists mainly of water (71.2 ± 0.5%), crude protein (14.2 ± 0.3%) and smaller amounts of fat (5.9 ± 0.2%) and carbohydrates (7.3 ± 0.1% digestible carbohydrates and 1.6 ± 0.3% fibre – mainly chitin). In the dried forms, due to the low-water content (4.9 ± 1.2%), crude protein (46.5 ± 1.5%), fat (25.8 ± 7.1%) and carbohydrates (17.1 ± 8.8% digestible carbohydrates and 3.8 ± 1.1% dietary fibre – mainly chitin) are correspondingly higher. The energy value of TM frozen is 135.8 ± 3.1 kcal/100 g of NF, whereas of the dried NF forms is 485.9 ± 40.6 kcal/100 g of NF. Analytical data on the amino acid composition (Appendix A), the fatty acid profile (Appendix B), antinutrients (Table 18), minerals and metals (Appendices D and E) and vitamins (Appendices F–H) have been provided for several batches of the NF forms.

3.9.1 | Protein content and protein quality

The frozen and dried forms of the NF contain on average 14.2 and 46.5 g crude protein per 100 g, respectively, calculated using the conventional nitrogen-to-protein conversion factor of 6.25 (Table 1). The Panel notes that the use of this conventional factor overestimates the true protein content in the NF due to the presence of non-protein nitrogen derived mainly from chitin (Janssen, Vincken, et al., 2017). Based on the amino acid profile of yellow mealworms, Janssen, Vincken, et al. (2017) proposed a nitrogen-to-protein conversion factor of 4.76. Using this factor, the true protein content of the NF amounts to an average of 10.8 g/100 g for the TM frozen and 35.4 g/100 g for the dried forms. For regulatory purposes (i.e. nutritional labelling), protein is defined as the total nitrogen measured by the Kjeldahl method multiplied by a nitrogen-to-protein conversion factor of 6.25 [Regulation (EU) No 1169/2011 on the provision of food information to consumers].

The applicant quantified the amino acids in TM frozen, TM dried and TM powder (five batches analysed per NF form) according to ISO 13903:2005 and/or Commission Regulation (EC) No 152/2009.[18] The results for the amount of individual amino acids in 100 g of the NF are reported in Appendix A.

The applicant attempted to investigate the protein quality for all three NF forms (five independently produced batches per form) by using an in vitro method used for the determination of soluble nitrogen content in animal feeding stuffs after treatment with pepsin in diluted hydrochloric acid (ISO 6655:1997). The Panel notes that estimates of soluble nitrogen cannot be used in scoring systems such as the Protein Digestibility-Corrected Amino Acid Score (PDCAAS) and the Digestible Indispensable Amino Acid Score (DIAAS) to quantify protein quality in line with the standards set by FAO and IAEA (2024).

As summarised by EFSA NDA Panel (2021a), Jensen et al. (2019) reported a PDCAAS of 76% for freeze-dried yellow mealworm, based on true faecal crude protein digestibility in rats and the amino acid reference profile for children aged 0.5–3 years (FAO, 2013). In a previous assessment by the EFSA NDA Panel of the safety of frozen and dried formulations from whole yellow mealworm as a novel food, the true ileal protein digestibility of dried yellow mealworm was reported to be 64% using a dynamic in vitro gastrointestinal model (tiny-TIM), and the DIAAS corresponded to 51%, with sulfur amino acids (methionine + cysteine) as the limiting ones, calculated using the FAO reference patterns for individuals above 3 years (EFSA NDA Panel, 2021b). Malla et al. (2022) calculated the DIAAS values for dried, ground yellow mealworm, through the ileal digestibility of indispensable amino acids measured in growing pigs. The DIAAS values, calculated also using the FAO reference patterns, increased with age across population groups, corresponded to 45% for infants, 54% for young children and 64% for older children, adolescents and adults with sulfur amino acids (methionine + cysteine) as the limiting ones. In a more recent study, Hammer et al. (2023) investigated the DIAAS of various forms of yellow mealworm (whole blanched, whole blanched and freeze-dried, and whole blanched, freeze-dried and ground), across age groups from infants (up to 6 months) to adults, employing an in vitro model reportedly based on the INFOGEST digestion protocol. The in vitro total protein digestibility, calculated based on total amino acids (true protein), ranged from approximately 94% to 99% for the tested forms of whole yellow mealworm. For infants, tryptophan was the limiting amino acid, with DIAAS values lowest in powdered forms (around 40% considering the reported standard deviation) and highest in freeze-dried forms (around 78% considering the reported standard deviation). For children aged 6 months to 3 years, sulfur amino acids were limiting in all forms, with DIAAS values ranging from approximately 74 to 95% (considering the reported standard deviations). For individuals over 3 years of age, sulfur amino acids were also the limiting ones across all forms, with DIAAS values ranging from about 90% to 112% (considering the reported standard deviations).

The Panel notes that heterogeneous information is available on the protein quality of yellow mealworm forms. In line with what the Panel had previously concluded (2021a), this heterogeneity may be caused by the differences in the processing of the source, the different techniques and models employed to assess protein digestibility, as well as the quantitative difference between the crude and 'true' protein content.

Provided that the NF would not be the sole source of dietary protein, that it is integrated into a varied and mixed diet and considering that the average protein intake in the EU population is high and frequently above the dietary reference values (DRVs) (EFSA NDA Panel, 2012), the consumption of the NF is not expected to negatively impact protein nutrition.

[18] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. OJ L 54, 26.2.2009, pp. 1–130.

3.9.2 | 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 23.51%–24.37%, 32.53%–35.55% and 40.63%–42.85% of the total fatty acids, respectively. The average trans-fatty acid content is 0.05% of total fatty acids.

The applicant provided analytical data on the levels of some minerals and vitamins (Appendices D–H).

Considering the highest values reported in Appendices D–H, the specifications (Table 14) and the estimated P95 of exposure to the NF, the Panel notes that none of the existing upper levels for the analysed micronutrients are expected to be exceeded from the NF intake alone, for any population group.

The concentration of Mn in the NF according to specifications (Table 14), may reach up to 11 mg/kg. The EFSA NDA Panel (2023) established a safe level of intake of Mn of 8 mg/day for adults ≥ 18 years, ranging between 2 and 7 mg/day for other population groups.

The NDA Panel estimated the intake of Mn from the NF, considering the product specifications for Mn (Table 14) and the estimated daily intake of the NF for all population groups (Table 17). The highest estimated P95 intake of Mn from the NF ranges from 0.086 mg/day in infants to 0.394 mg/day in adults. As compared to the safe levels of intake established by the EFSA NDA Panel (2023), the daily intake of Mn from the NF accounts for 1.46% of the aforementioned safe level of intake for young children, 2.67% for other children, 2.83% for adolescents and 4.93% for adults. The Panel considers that such Mn intake (< 5% of the safe levels of intake[19]) is not of concern. Additionally, the Panel notes that an increasing number of NFs deriving from *T. molitor* larvae becomes available to the EU consumers. It is expected that the combined intake of products from *T. molitor* larvae may increase the intake of manganese.

3.9.3 | Antinutrients

Insects may contain antinutritional factors such as tannins, oxalates, phytates, hydrogen cyanide (Meyer-Rochow et al., 2021; Shantibala et al., 2014), thiaminases (Nishimune et al., 2000) and protease inhibitors (Eguchi, 1993). Based on the literature findings, the applicant determined the concentrations of phytic acid, total polyphenols, oxalates, trypsin inhibitors and hydrocyanic acid in five independently produced batches of TM powder (Table 18). The reported values in the NF are comparable to the occurrence levels of these compounds in other foodstuffs (EFSA CONTAM Panel, 2019; Gupta, 1987; Holmes & Kennedy, 2000; Rao & Prabhavathi, 1982; Schlemmer et al., 2009), and also to other published EFSA *T. molitor* opinions (EFSA NDA Panel, 2021a, 2021b, 2021c). Moreover, the Panel notes that not all polyphenols exhibit antinutrient activity.

The NF contains on average 6.5 (± 0.8) g chitin in 100 g of NF (dried form). Regarding the nutritional impact of chitin, 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, although partial digestion in the human stomach has been suggested (Muzzarelli et al., 2012; Paoletti et al., 2009). 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 (Anastopoulos et al., 2017; Franco et al., 2004) possibly negatively affecting their bioavailability (Baye et al., 2017).

TABLE 18 Batch-to-batch analysis of antinutrients in TM powder.

	Batch number					
Parameter (unit)	**#11**	**#12**	**#13**	**#14**	**#15**	**Analytical method**
Phytic acid (g/100 g)	< 0.14	< 0.14	< 0.14	< 0.14	< 0.14	Analytical Biochemistry Vol. 77:536–539 (1977)
Total polyphenols (mg/kg expressed as gallic acid)	6510	6100	5830	6130	5560	Internal method, Spectrophotometry
Oxalic acid (oxalates) (g/100 g)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	Internal, IC-EC
Trypsin Inhibitor Activity (mg/g)	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	NEN-EN-ISO 14902:2001
Total hydrocyanic acid (mg/kg)	2.1	1.6	4.3	1.5	1.6	Internal method, HS-GC-NPD

Abbreviations: HS-GC-NPD, Headspace gas chromatography/nitrogen–phosphorus detector; IC-EC, ion chromatography/conductivity detection.

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

[19]As reported by the published minutes of the 154th meeting of the working group on novel foods (WG NF 2024), the WG considers that 'for the purpose of the assessment of NFs, intakes that lead to a significant increase of Manganese (Mn) intake as compared to the safe levels set by the EFSA NDA Panel (2023) are considered of concern. In the absence of adequate data to establish an UL, the NDA Panel (2023) established the safe levels of intake of Mn based on observed background intake of Mn among high consumers from the general population (P95 estimates). Based on experts' judgement and criteria set by the WHO/FAO's Codex Alimentarius Commission (2015) for selecting foods/food groups that contribute significantly to total dietary exposure of a contaminant or toxin, the WG concluded that Mn intake from the NF exceeding 5% of the highest P95 background dietary intake is considered as a significant contribution'.

3.10 | Toxicological information

The Panel notes that no toxicological studies with the NF were provided. The toxicological profile of *T. molitor* larvae has been previously assessed by the Panel (EFSA NDA Panel, 2021a, 2021b, 2023). The Panel noted that *T. molitor* larvae should be reared separately from the adults since it has been reported that *T. molitor* adults may excrete potentially toxic substances as part of their defence mechanisms (Attygalle et al., 1991; Brown et al., 1992; Ladisch et al., 1967). The Panel also assessed toxicological studies available in the literature (in vitro and in vivo genotoxicity, acute, subacute and subchronic toxicity) with processed (freeze-dried) *T. molitor* larvae as the testing material (Han et al., 2014, 2016). The Panel concludes that the material assessed in these studies can be considered representative of the NF only with regards to the profile of the endogenously produced compounds of possible concern but not for any compounds that can be present due to the rearing conditions (e.g. feed) or processing (EFSA NDA Panel et al., 2021a, 2021b). Taking into account the production process and the nature of the NF, the Panel considers that no additional toxicological studies are required on the NF.

3.11 | Allergenicity

The Panel has previously considered that the consumption of the NF source (yellow mealworm), may trigger primary sensitisation to yellow mealworm proteins. The Panel has also considered that allergic reactions may occur in subjects allergic to crustaceans, dust mites and molluscs due to cross-reactivity. Furthermore, the Panel has noted that additional allergens may end up in the NF, if these allergens are present in the substrate fed to the insects (e.g. gluten). This may include allergens listed in the Annex II of Regulation (EU) No 1169/2011 (EFSA NDA Panel, 2021a, 2021b, 2023). The Panel notes that allergic reactions may occur upon consumption.

4 | DISCUSSION

The NF which is the subject of the application comprises the frozen, dried and powder forms of the yellow mealworm (*T. molitor* larva). The production process is sufficiently described and does not raise safety concerns. The Panel considers that the NF is sufficiently characterised. The composition of TM dried/TM powder differs from the one of TM frozen due to the reduced water content in the dried forms. The frozen form mainly consists of water, crude protein, carbohydrates and fat, whereas the dried forms consist of crude protein, fat and carbohydrates. The concentrations of contaminants in the NF may depend mainly on their occurrence in the insect feed. Provided that applicable EU legislation regarding feed is followed, the consumption of the NF does not raise safety concerns.

The Panel notes that, despite the high variability noted in the oxidative parameters (*p*-anisidine and free-fatty acids values), there are no safety concerns regarding stability if the NF complies with the proposed specification limits during its entire shelf life. The Panel considers that the data provided sufficient information with respect to the stability of the NF with a shelf life of 6 months.

The applicant intends to market the NF as an ingredient in several food products. The target population is the general population. Intake was estimated based on the use of the NF as an ingredient in the intended food categories at the maximum proposed levels across surveys in the EFSA Comprehensive European Food Consumption Database. The highest intake estimate (based on the scenario of inclusion of the highest amount of NF dry matter) was calculated for young children (1–< 3 years old), ranging from 78 to 883 mg NF/kg bw per day at the 95th percentile of the intake distribution.

The Panel notes that consumption of the NF under the proposed uses and use levels is not expected to negatively impact protein nutrition, and also does not contribute substantially to the total dietary exposure of the analysed undesirable substances.

None of the existing upper levels for the analysed micronutrients are expected to be exceeded considering the proposed uses and use levels from the NF intake alone, for any population group. The reported concentrations of antinutritional factors in the NF are similar to those in other foods. The Panel considers that chitin is not expected to be digested in the small intestine of humans to any significant degree and is assumed to be excreted mainly unchanged. 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. No safety concerns arise from the history of use and toxicological information of yellow mealworm, or from the compositional data of the NF. The Panel considers that the consumption of the NF may trigger primary sensitisation and allergic reactions to yellow mealworm proteins. The Panel also considers that allergic reactions may occur in subjects allergic to crustaceans, mites and molluscs (cross-reactivity). Additionally, the Panel notes that allergens from the feed (e.g. gluten) may be present in the NF.

5 | CONCLUSIONS

The Panel concludes that the NF is safe under the proposed uses and use levels. The Panel notes that allergic reactions may occur upon consumption.

6 | RECOMMENDATION

As previously recommended by the Panel (EFSA NDA Panel, 2021a, 2021b, 2023), research should be undertaken on the allergenicity to yellow mealworm, including cross-reactivity to other allergens.

7 | STEPS TAKEN BY EFSA

1. On 2/12/2019, EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of frozen and dried forms of whole yellow mealworm (*Tenebrio molitor*) as a novel food Ref.Ares 20197551669.
2. On 2/12/2019, a valid application on the safety of frozen and dried forms of whole yellow mealworm (*Tenebrio molitor*), which was submitted by name of the company, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2018/0396) and the scientific evaluation procedure was initiated.
3. On 29/04/2020, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4. On 10/11/2021, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5. On 22/12/2023, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
6. On 18/04/2024, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
7. On 11/09/2024, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
8. On 28/10/2024, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
9. During its meeting on 28/11/2024, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of frozen and dried forms of whole yellow mealworm (*Tenebrio molitor*) as a NF pursuant to Regulation (EU) 2015/2283.

ABBREVIATIONS

3-MCPD	3-monochloropropane-1,2-diol
ADF	acid detergent fibre
ADL	acid detergent lignin
ADME	absorption, distribution, metabolism and excretion
AFNOR	Association Française de Normalisation
AOAC	Association of Official Analytical Chemists
ASU	Official Collection of Analysis Methods According To § 64 of the German Food And Feed Code (LFGB)
a_w	water activity
BiiF	Belgian Insect Industry Federation
BIOHAZ	EFSA Panel on Biological Hazards
BRD	Bacteriology Reference Department
CFU	colony forming units
CONTAM	EFSA Panel on Contaminants in the Food Chain
DIAAS	Digestible Indispensable Amino Acid Score
DietEx	Dietary Exposure tool
DRVs	dietary reference values
EN	Europaische Norm (European Standard)
FAO	Food and Agriculture Organization of the United Nations
GC–MS/MS	gas chromatography with tandem mass spectrometry
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Points
HPLC-DAD	high-performance liquid chromatography with diode array detection
HPLC-FLUO	high-performance liquid chromatography -fluorescence detection
HS-GC-NPD	Headspace gas chromatography/nitrogen–phosphorus detector
IAA	indispensable amino acids
IAEA	International Atomic Energy Agency
IC-EC	ion chromatography/conductivity detection
IC-PAD	ion chromatography-pulsed amperometric detection
ICP-MS	inductively coupled plasma-mass spectrometry
ISO	International Organization for Standardization
LC-DAD	liquid chromatography-diode array detection

LC–MS/MS	liquid chromatography–tandem mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
MLs	maximum levels
Mn	manganese
MUFA	monounsaturated fatty acids
N.D.	not detected
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
ndl-PBCs	non dioxin-like polychlorinated biphenyls
NMKL	Nordic-Baltic Committee on Food Analysis
P95	95th percentile
PA	*p*-anisidine value
PAHs	polycyclic aromatic hydrocarbons
PCBs	polychlorinated biphenyls
PCDD/F TEQ	polychlorinated dibenzo-para-dioxins and polychlorinated dibenzofurans
PCR	polymerase chain reaction
PDCAAS	Protein Digestibility-Corrected Amino Acid Score
PFAS	perfluoroalkyl substances
PFHxS	Perfluorhexanesulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulphonic acid
PUFA	polyunsaturated fatty acids
SCF	Scientific Committee on Food
SD	standard deviation
TIM	TNO gastro-Intestinal Model
UB	upper-bound
US FDA/CFSAN	United States Food and Drug Administration/the Center for Food Safety and Applied Nutrition
WHO (2005)PCDD/F + PCB TEQ	sum of polychlorinated dibenzo-p-dioxins-polychlorinateddibenzofurans-polychlorinated biphenyls expressed as World Health Organization toxic equivalents
WHO	World Health Organization

REQUESTOR

European Commission

QUESTION NUMBER

EFSA-Q-2018-00746

PANEL MEMBERS

Dominique Turck, Torsten Bohn, Jacqueline Castenmiller, Stefaan De Henauw, Montaña Cámara, Karen Ildico Hirsch-Ernst, Ángeles Jos, Alexandre Maciuk, Inge Mangelsdorf, Breige McNulty, Androniki Naska, Kristina Pentieva, Alfonso Siani and Frank Thies.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

Batch-to-batch amino acid analysis of the NF

Amino acid (g/100 g product)	TM frozen					TM dried					TM powder				
	#112	#113	#114	#115	#116	#117	#118	#119	#26	#27	#93	#94	#95	#96	#97
Alanine	1.33	1.25	1.51	1.56	1.44	3.51	3.49	3.56	4.06	3.60	3.81	3.86	3.77	3.81	3.76
Arginine	0.83	0.80	1.14	1.10	1.04	2.49	2.52	2.42	2.82	2.64	2.86	2.74	2.78	2.93	2.75
Aspartic acid	1.46	1.39	1.77	1.68	1.55	3.82	3.86	3.89	4.45	4.15	4.33	4.43	4.38	4.50	4.40
Glutamic acid	2.14	2.07	2.34	2.24	2.08	5.39	5.66	5.36	5.73	5.41	6.04	6.10	6.08	6.07	6.00
Glycine	1.01	0.95	1.09	1.10	1.03	2.51	2.51	2.50	2.85	2.60	2.74	2.73	2.68	2.71	2.66
Histidine*,[a]	0.66	0.61	0.65	0.64	0.61	1.48	1.46	1.47	1.67	1.53	1.62	1.62	1.60	1.64	1.61
Hydroxyproline	/	/	< 0.2 (LOQ)	< 0.2 (LOQ)	< 0.2 (LOQ)	/	/	/	< 0.2 (LOQ)	< 0.2 (LOQ)	< 0.2 (LOQ)	< 0.2 (LOQ)	< 0.2 (LOQ)	< 0.2 (LOQ)	< 0.2 (LOQ)
Isoleucine*,[a]	0.78	0.77	0.89	0.84	0.80	2.05	2.03	2.08	2.30	2.14	2.22	2.25	2.24	2.30	2.25
Leucine*,[a]	1.23	1.18	1.54	1.49	1.40	3.41	3.38	3.46	4.00	3.70	3.82	3.86	3.83	3.90	3.81
Lysine*,[a]	1.08	1.01	1.12	1.05	1.01	2.51	2.52	2.54	3.08	2.88	3.02	3.08	3.05	3.05	3.08
Ornithine	/	/	< 0.05 (LOQ)	< 0.05 (LOQ)	< 0.05 (LOQ)	/	/	/	0.15	0.12	0.17	< 0.05 (LOQ)	< 0.05 (LOQ)	< 0.05 (LOQ)	< 0.05 (LOQ)
Phenylalanine*,[a]	1.12	0.91	0.77	0.73	0.68	1.37	1.59	1.48	1.97	1.82	1.87	1.89	1.93	2.00	1.95
Proline	1.24	1.10	1.44	1.33	1.32	3.23	3.32	3.05	3.40	3.11	3.81	3.52	3.43	3.73	3.62
Serine	0.80	0.75	0.98	0.98	0.91	2.18	2.17	2.16	2.48	2.27	2.34	2.46	2.42	2.43	2.42
Threonine*,[a]	0.73	0.69	0.86	0.83	0.78	1.86	1.90	1.87	2.13	1.97	2.08	2.14	2.11	2.13	2.09
Tyrosine	1.93	1.88	1.44	1.43	1.38	3.29	3.12	3.33	3.74	3.32	3.56	3.53	3.50	3.56	3.46
Valine*,[a]	0.96	0.92	1.27	1.25	1.18	2.89	2.77	2.90	3.41	3.12	3.25	3.22	3.20	3.24	3.17
Cysteine	0.19	0.17	0.17	0.19	0.19	0.46	0.46	0.42	0.45	0.43	0.50	0.39	0.37	0.41	0.41
Methionine*,[a]	0.23	0.21	0.22	0.26	0.28	0.61	0.66	0.64	0.66	0.64	0.58	0.62	0.59	0.66	0.68
Tryptophan*,[a]	0.23	0.17	0.24	0.23	0.23	0.58	0.59	0.57	0.60	0.58	0.58	0.61	0.61	0.60	0.60

Note: '/', not analysed.

Abbreviation: LOQ, limit of quantification.

[a]ISO 13903:2005.

*Essential amino acids.

APPENDIX B

Batch-to-batch analysis of fatty acids in the NF forms

Fatty acids (g/100 g NF)	TM frozen					TM dried					TM powder				
	#1	#2	#3	#4	#5	#41	#120	#121	#122	#123	#11	#12	#13	#14	#15
Total Saturated fats (SFAs)	1.37	1.38	1.39	1.27	1.35	6.61	7.79	8.13	4.89	7.73	5.07	4.50	4.43	4.51	4.61
C4:0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C6:0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C7:0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C8:0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C9:0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C10:0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C12:0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.09	0.077	0.088	0.053	0.801	0.05	0.03	0.03	0.03	0.04
C13:0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.02	0.013	0.012	0.0079	0.013	0.01	< 0.01	< 0.01	< 0.01	< 0.01
C14:0	0.11	< 0.01	0.11	0.10	0.11	1.24	0.849	0.867	< 0.06	0.862	0.55	0.38	0.37	0.37	0.43
C15:0	0.01	0.01	0.01	0.01	0.01	0.03	0.039	0.38	0.023	0.389	0.05	0.04	0.04	0.04	0.04
C16:0	0.99	1.00	1.01	0.92	0.98	4.45	5.66	5.97	3.59	5.65	3.70	3.32	3.21	3.27	3.33
C17:0	0.04	0.04	0.04	0.04	0.04	0.09	0.043	0.042	0.26	0.418	0.05	0.05	0.13	0.13	0.13
C18:0	0.22	0.22	0.21	0.20	0.21	0.66	0.963	0.97	0.578	0.909	0.62	0.63	0.63	0.64	0.62
C20:0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.03	0.046	0.044	0.026	0.038	0.02	0.02	0.02	0.02	0.02
C22:0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C24:0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	0.02	0.03	< 0.01	< 0.01	< 0.01
MUFA	1.80	32.09	31.55	31.79	31.78	12.27	15.4	15.8	9.53	15.3	7.67	6.04	5.97	32.53	33.65
C11: 1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	/	/	/	/	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C12: 1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C13: 1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	/	/	/	/	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C14:1 (n-5c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C14: 1 (n-5t)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	/	/	/	/	< 0.01	0.01	< 0.01	< 0.01	< 0.01
C15:1 (n-5 c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C15:1 (n-5t)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	/	/	/	/	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C16:1 (n-7c)	0.06	0.06	0.06	0.05	0.06	0.56	0.626	0.647	0.395	0.211	0.30	0.20	0.19	0.20	0.23
C16:1 (n-7 t)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	/	/	/	/	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C17:1 (n-7c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C17:1 (n-7t)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	/	/	/	/	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C18:1 (n-6c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

(Continued)

Fatty acids (g/100 g NF)	TM frozen					TM dried					TM powder				
	#1	#2	#3	#4	#5	#41	#120	#121	#122	#123	#11	#12	#13	#14	#15
C18: 1 (n-7c)	0.02	0.02	0.02	0.02	0.02	0.06	0.108	0.107	0.63	0.109	0.08	0.08	0.08	0.08	0.08
C18: 1 (n-7 t)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C18:1 (n-9)	1.71	1.74	1.73	1.60	1.70	11.62	14.4	14.8	8.9	14.3	7.25	5.72	5.63	5.68	6.14
C18:1 (n-9 t) + C18:1 (n-12 t)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	/	/	/	< 0.01	< 0.01	0.01	0.01	0.01
C20:1 (n-9c)	0.01	0.01	0.01	0.01	0.01	0.04	0.391	0.042	0.25	< 0.08	0.03	0.04	0.04	0.04	0.04
C22: 1 (n-11)	< 0.01	< 0.01	< 0.01	0.01	0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C22: 1 (n-9 c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	0.02	0.02	0.02
C22: 1 (n-9 t)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	/	/	/	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C24:1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
PUFA (PUFAs)	43.99	43.27	43.85	43.77	43.64	7.41	9.25	9.47	5.77	9.510	8.76	7.9	7.85	42.70	42.12
C18: 2 (9c, 11t)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	/	/	/	/	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C18: 2 (n-6c)	2.39	2.34	2.41	2.20	2.33	7.17	8.43	< 0.08	5.37	8.79	8.27	7.44	7.42	7.46	7.70
C18: 2 (n-6t)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C18: 2 t2	0.01	0.01	0.01	0.01	0.01	0.06	/	/	/	/	0.05	0.04	0.04	0.04	0.04
C20: 2 (n-6c)	< 0.01	0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.02	0.021	0.12	0.021	0.02	0.02	0.02	0.02	0.02
C22:2 (n-6c)	< 0.01	0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C18:3 (n-3)	0.12	0.12	0.13	0.11	0.12	0.17	0.78	0.566	0.384	0.684	0.45	0.41	0.41	0.42	0.42
C18:3 (n-6)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C18:3 t3 (C18:3 t1 + C18:3 t2)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	/	/	/	/	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C20:3 (n-3c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C20:3 (n-6c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C18:4 (n-3)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C20:4 (n-6c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.03	< 0.08	< 0.08	< 0.06	< 0.08	0.03	0.03	< 0.01	< 0.01	< 0.01
C20:5 (n-3c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C22:5 (n-3c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C22:5 (n-6c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C22:6 (n-3c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	< 0.08	< 0.06	< 0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C22:3 (n-3c) + C22:4 (n-6c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.04	/	/	/	/	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

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Fatty acids (g/100 g NF)	TM frozen					TM dried					TM powder				
	#1	#2	#3	#4	#5	#41	#120	#121	#122	#123	#11	#12	#13	#14	#15
C20:1 (n-9 t) + C18:2 (10 t,12c) + C20:1 (n-15c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	/	/	/	/	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Total Trans	0.01	0.01	0.27	0.22	0.28	0.06	0.025	0.018	0.125	0.025	0.05	0.05	0.29	0.28	0.27
Total omega-3	0.12	0.12	2.16	2.12	2.13	0.17	0.787	0.571	0.386	0.693	0.45	0.41	2.25	2.27	2.18
Total omega-6	2.39	2.34	41.57	41.53	41.39	7.20	0.846	8.90	5.380	8.810	8.30	7.47	40.47	40.31	39.82
Other fatty acids	< 0.01	< 0.01	< 0.05	< 0.05	< 0.05	< 0.01	/	/	0.226	0.349	< 0.01	< 0.01	< 0.05	< 0.05	< 0.05
Omega-6/ Omega-3 Ratio	19.24	19.13	19.23	19.62	19.43	42.04	0.01	0.016	1.39	12.71	18.58	18.38	17.97	17.79	18.25

APPENDIX C

Batch-to-batch analytical data on dioxins and dioxin-like PCBs in the TM powder

	Batch number					
Parameters (μg/kg)	**#11**	**#12**	**#13**	**#14**	**#15**	**Analytical method**
PAHs						Internal method, LC–MS/MS
Acenaphthene	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	
Acenaphthylene	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	
Anthracene	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	
Benzo(k)fluoranthene	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	
Benzo(g,h,i)perylene	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	
Dibenz(a,h)anthracene	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	
Fluoranthene	1.2	1.1	1.1	1.1	1.2	
Fluorene	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	
Indeno(1,2,3-cd)pyrene	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	
Naphthalene	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	
Phenanthrene	7.0	6.7	6.5	7.9	6.8	
Pyrene	0.92	0.96	0.83	0.78	0.97	
Sum PAH 4	< 0.50	< 0.50	< 0.5	< 0.5	< 0.5	
Sum of all positive identified PAH	9.1	8.8	8.4	9.7	9.0	
Dioxins (pg/g fat)						
PCDD/F + PCB TEQ (lower-bound)	4.10	4.03	2.94	4.29	3.48	Conform EC 2017/644 (food) and EC 2017/771 (feed)
PCDD/F + PCB TEQ (medium-bound)	4.19	4.12	3.03	4.41	3.55	
2,3,7,8-TetraCDD	< 0.0692	< 0.0712	< 0.0699	< 0.0889	< 0.0554	
1,2,3,7,8-PentaCDD	< 0.0692	< 0.0712	< 0.0699	< 0.0889	< 0.0554	
1,2,3,4,7,8-HexaCDD	< 0.0692	< 0.0712	< 0.0699	< 0.0889	< 0.0554	
1,2,3,6,7,8-HexaCDD	0.753	0.839	0.798	0.903	0.648	
1,2,3,7,8,9-HexaCDD	0.129	0.115	0.142	0.142	0.114	
1,2,3,4,6,7,8-HeptaCDD	14.8	14.1	13.9	14.8	13.6	
OctaCDD	56.3	60.7	55.6	57.5	58.4	
2,3,7,8-TetraCDF	0.470	0.315	0.602	0.698	0.255	
1,2,3,7,8-PentaCDF	0.166	0.177	0.189	0.216	0.142	
2,3,4,7,8-PentaCDF	0.391	0.366	0.385	0.433	0.291	
1,2,3,4,7,8-HexaCDF	0.838	0.957	0.917	1.02	0.797	
1,2,3,6,7,8-HexaCDF	5.58	3.94	0.676	0.733	2.43	
1,2,3,7,8,9-HexaCDF	< 0.0692	< 0.0712	< 0.0699	< 0.0889	< 0.0554	
2,3,4,6,7,8-HexaCDF	0.140	0.116	0.180	0.196	0.102	
1,2,3,4,6,7,8-HeptaCDF	6.15	5.71	5.63	6.00	5.14	
1,2,3,4,7,8,9-HeptaCDF	0.256	0.195	0.193	0.252	0.135	
OctaCDF	4.68	4.53	4.27	4.06	2.25	
PCDD/F TEQ (lower-bound)	1.14	0.963	0.668	0.734	0.733	
PCDD/F TEQ (medium-bound)	1.22	1.04	0.745	0.832	0.794	
PCB 77	29.7	27.5	29.4	31.0	26.3	Conform EC 2017/644 (food) and EC 2017/771 (feed)
PCB 81	< 0.865	< 0.890	< 0.874	< 1.11	< 0.693	
PCB 105	485	507	486	494	437	
PCB 114	< 17.3	18.4	< 17.5	< 22.2	16.8	
PCB 118	3520	3820	3840	3880	2930	
PCB 123	39.1	41.9	196	55.1	26.6	
PCB 126	27.6	28.5	20.5	33.3	25.8	

(Continues)

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	Batch number					
Parameters (μg/kg)	**#11**	**#12**	**#13**	**#14**	**#15**	**Analytical method**
PCB 156	1500	1630	1630	1680	1180	
PCB 157	137	138	128	144	111	
PCB 167	788	866	873	866	619	
PCB 169	< 0.865	< 0.890	< 0.874	< 1.11	< 0.693	
PCB 189	197	201	207	217	155	
PCB TEQ (lower-bound)	2.96	3.07	2.27	3.56	2.74	
PCB TEQ (medium-bound)	2.97	3.08	2.28	3.57	2.75	
PCB TEQ (upper-bound)	2.99	3.10	2.30	3.59	2.76	
Total 6 ndl-PCB (lower-bound)	60.9	68.1	69.5	68.7	47.3	
Total 6 ndl-PCB (medium-bound)	60.9	68.1	69.5	68.7	47.3	
Total 6 ndl-PCB (upper-bound)	60.9	68.1	69.5	68.7	47.3	
Furan (μg/kg)	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0	US FDA/CFSAN 2006–10; mod.
2-MCPD (2-Monochloropropandiol)	< 5	< 5	< 5	< 5	< 5	Internal Method, GC–MS/MS

Abbreviations: GC–MS/MS, gas chromatography–tandem mass spectrometry; LC–MS/MS, liquid chromatography–tandem mass spectrometry; PAHs, polycyclic aromatic hydrocarbons; PCBs, polychlorinated biphenyls; PCDDs, polychlorinated-p-dioxins.

APPENDIX D

Batch-to-batch analysis of minerals and metals in the TM frozen, on a product basis

	Batch number									
Minerals (mg/100 g)	**#1**	**#2**	**#3**	**#4**	**#5**	**#18**	**#19**	**#31**	**#32**	**#33**
Calcium	21	/	14	15	13	14	14	/	/	/
Copper	0.9	0.89	/	/	/	/	/	0.97	0.91	0.93
Iron	1.9	/	2.3	2.2	2.5	2.3	2.7	/	/	/
Magnesium	120	/	89	92	90	89	87	/	/	/
Manganese	0.41	0.4	/	/	/	/	/	0.47	0.44	0.44
Phosphorus	320	/	280	290	290	280	270	/	/	/
Potassium	330	/	330	340	330	330	320	/	/	/
Sodium	/	/	69	59	63	69	67	/	/	/
Zinc	4.7	4.7	/	/	/	/	/	5	4.9	4.9
Selenium	0.024	0.022	/	/	/	/	/	0.028	0.026	0.024
Molybdenum	0.038	0.038	/	/	/	/	/	0.04	0.038	0.037

Note: Analytical method: Internal method (digestion according NEN-EN 13805), ICP–MS. '/': not analysed.

APPENDIX E

Batch-to-batch analysis of minerals in the TM dried and TM powder, on a product basis

	Batch number																			
	TM dried										TM powder									
Minerals (mg/100 g)	#112	#113	#114	#117	#124	#125	#126	#127	#128	#129	#11	#12	#13	#14	#15	#130	#131	#132	#133	#134
Calcium	54	46	49	/	56	/	/	/	63	/	49	50	51	36	51	/	/	/	/	/
Iron	3.9	4.1	/	/	6.3	/	/	/	6.4	/	5.3	5.7	5.8	3.1	5.8	/	/	/	/	/
Magnesium	26	186	190	/	320	/	/	/	300	/	290	300	310	170	320	/	/	/	/	/
Manganese	/	/	/	1.1	/	1.0	1.1	1.1	/	1.098	/	/	/	/	/	1.1	1.0	1.1	1.1	1.0
Phosphorus	642.6	568	586.6		< 10	/	/	/	/	/	920	950	990	< 10	< 10	/	/	/	/	/
Potassium	738.9	585	612	/	1100	/	/	/	/	/	1100	1100	1200	1200	1100	/	/	/	/	/
Sodium	117	108	110	/	/	/	/	/	/	/	2030	2060	2160	1940	2150	/	/	/	/	/
Selenium	/	/	/	0.61	/	0.56	0.61	0.71	/	0.56	/	/	/	/	/	0.63	0.61	0.65	0.64	0.62

Note: '/', not analysed.

APPENDIX F

Batch-to-batch analysis of vitamins in TM frozen

Vitamin (unit)	#112	#1	#2	#3	#4	#5	#112	#113	#114	#115	Analytical method
Vitamin B12 (µg/100 g)	/	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	/	/	/	/	J. AOAC 2008, vol 91 No 4
Folate (µg/100 g)	/	/	/	/	/	51.1	50	58.1	50.6	44	NMKL 111:1985
Niacin (mg/kg)	/	39.4	38.9	38.2	38	42.2	/	/	/	/	EN 15652:2009
Pyridoxine (mg/100 g)	/	/	/	/	/	0.195	0.188	0.207	0.187	0.177	EN 14164:2014
Riboflavin (mg/100 g)	0.141	0.117	0.135	0.123	0.127	/	/	/	/	/	EN 14152:2014 mod.
Thiamin (mg/100 g)	/	0.144	0.14	0.151	0.146	0.124	/	/	/	/	EN 14122: 2014 mod.
Retinol (vitamin A) (µg/100 g)	/	< 21	< 21	< 21	< 21	< 21	/	/	/	/	EN 12823–12014
Vitamin D3 (Cholecalciferol) (µg/100 g)	/	/	/	/	/	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	EN 12821:2009
Alpha-tocopherol (mg/100 g)	/	0.191	0.171	0.21	0.21	0.179	0.42	/	/	/	EN 12822:2014
Vitamin C (mg/100 g)	/	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	/	/	/	/	ISO 20635:2018

Note: '/', not analysed.

APPENDIX G

Batch-to-batch analysis of vitamins in TM powder

		Batch number TM powder					
Vitamin (unit)	**Unit**	**#11**	**#12**	**#13**	**#14**	**#15**	**Analytical method**
Vitamin B3 (niacin)	(mg/100 g)	11.1	12.4	12.6	12.8	11.9	EN 15652:2009
Vitamin B12 (cyanocobalamin)	µg/100 g)	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	J. AOAC 2008, vol 91 No 4
Vitamin C (ascorbic acid)	mg/100 g	< 0.5	< 0.5	< 0.5	< 0.5	1.92	ISO 20635:2018
Vitamin B1 (thiamin)	mg/100 g	0.408	0.444	0.467	0.451	0.389	EN 14122: 2014 mod.
Vitamin A (retinol)	µg/100 g	/	< 21	< 21	< 21	< 21	EN 12823–12014
Vitamin B2 (riboflavin)	(mg/100 g	0.405	0.322	0.337	0.36	0.413	EN 14152:2014 mod.
Vitamin E (alpha + gamma tocopherol)	mg/100 g	0.47	0.582	0.567	0.584	0.462	EN 12822:2014
		#130	**#131**	**#132**	**#133**	**#134**	
Vitamin B6 (pyridoxine)	mg/100 g	0.276	0.282	0.244	0.256	0.257	EN 14164:2014
Vitamin B9 (folate)	µg/100 g	115	117	122	87.6	87.5	NMKL 111:1985
Vitamin D3 (cholecalciferol)	µg/100 g	< 0.25	0.324	< 0.25	< 0.25	< 0.25	EN 12821:2009

Note: '/', not analysed.

APPENDIX H

Batch-to-batch analysis of vitamins in TM dried

	Batch number												
	Units	**#112**	**#113**	**#114**	**#117**	**#124**	**#125**	**#126**	**#127**	**#128**	**#129**	**#135**	**#136**	**Analytical method**
Vitamin B3 (niacin)	mg/100 g	7.18	6.49	6.63	11.5	10.9	/	/	/	/	/	/	/	USP 34, method 441, microb act. (first three) EN 15652:2009 (last two)
Vitamin B12 (cyanocobalamin)	µg/100 g	< 0.3	< 0.3	< 0.3	< 0.25	< 0.25	/	/	/	/	/	/	/	USP 39, method 171, microb. act. (first three) J. AOAC 2008, vol 91 No 4 (last two)
Vitamin B1 (thiamin)	mg/100 g	0.63	0.51	0.44	0.327	0.306	/	/	/	/	/	/	/	EN 14122: 2014 mod.
Vitamin E (alpha + gamma tocopherol)	mg/kg	< 10	< 10	< 10	/	/	/	/	/	/	/	/	/	HPLC-FLUO
Vitamin E (dl-alpha-tocopherol)	mg/100 g	/	/	/	/	/	/	/	/	/	/	0.472	0.609	EN 12822:2014
Vitamin A (retinol)	µg/100 g	/	/	/	/	/	/	/	/	/	/	< 21	< 21	EN 12823–12014
Vitamin B2 (riboflavin)	mg/100 g	0.84	0.73	0.74	/	/	/	/	/	0.79	0.78	/	/	HPLC
Vitamin C (ascorbic acid)	mg/kg	< 25	< 25	< 25	/	/	/	/	/	/	/	< 5	< 5	HPLC-DAD (first three) ISO 20635:2018 (last two)
Vitamin B6 (pyridoxine)	mg/100 g	/	/	/	0.249	0.242	0.237	0.27	0.244	/	/	/	/	EN 14164:2014
Vitamin B9 (folate)	µg/100 g	/	/	/	98.9	97.3	91.4	107	129	/	/	/	/	NMKL 111:1985
Vitamin D3 (cholecalciferol)	µg/100 g	/	/	/	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	/	/	/	/	EN 12821:2009

Note: '/': not analysed.

Abbreviations: HPLC-DAD, high-performance liquid chromatography with diode array detection; HPLC-FLUO, high-performance liquid chromatography -fluorescence detection.

ANNEX A

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

The Annex is available under the Supporting Information section of the online version of this output.