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# Safety of frozen and dried formulations from 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, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri, Marco Vinceti, Francesco Cubadda, Thomas Frenzel, Marina Heinonen, Rosangela Marchelli, Monika Neuhäuser-Berthold, Morten Poulsen, Miguel Prieto Maradona, Josef Rudolf Schlatter, Henk van Loveren, Ermolaos Ververis and Helle Katrine Knutsen

## Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on frozen and dried formulations from whole 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 *Tenebrio molitor*. The NF comprises the frozen and freeze-dried formulations of the yellow mealworm, whole or in the form of powder. The frozen formulation consists mainly of water, crude protein and fat whereas the freeze-dried formulations of crude protein, fat, digestible carbohydrates and fibre (chitin). The Panel notes that the levels of contaminants in the NF depend on the occurrence levels of these substances in the insect feed. The Panel notes furthermore 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 dried formulations of the NF have a high protein content, although the true protein levels in the NF are overestimated when using the nitrogen-to-protein conversion factor of 6.25, due to the presence of non-protein nitrogen from chitin. The applicant proposed to use the NF as whole frozen or whole dried insect, or in the form of powder, added as an ingredient to various food products such as cereal bars, pasta, meat imitates and bakery products. The target population is the general population. The Panel notes that, considering that the NF will not be the sole source of dietary protein, and the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous. The submitted toxicity studies from the literature did not raise safety concerns. The Panel considers that the consumption of the NF may induce primary sensitisation and allergic reactions to yellow mealworm proteins and may cause allergic reactions in subjects with allergy to crustaceans and dust mites. Additionally, allergens from the feed may end up in the NF. The Panel concludes that the NF is safe under the proposed uses and use levels.



**Keywords:** Novel foods, food safety, *Tenebrio molitor* larva, yellow mealworm, insect powder, entomophagy

**Requestor:** European Commission

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**Correspondence:** nda@efsa.europa.eu

**Panel members:** Dominique Turck, Torsten Bohn, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri and Marco Vinceti.

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# 1. Introduction

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

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

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

In accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission asks the European Food Safety Authority to provide a scientific opinion on the safety of whole and ground mealworms (*Tenebrio molitor*) larvae as a novel food.

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

In the process of the evaluation of this novel food, it became apparent that the Commission should amend the title of the mandate. The term "mealworm" is generic and does not exclusively refer to *Tenebrio molitor* larvae and therefore it should be replaced by "yellow mealworm". The term "whole and ground" indirectly implies that *Tenebrio molitor* powder may not be a product derived from using the whole insect. Moreover, the wording "frozen and dried formulations" would be a more inclusive descriptor for all three formulations of the novel food. On that basis, the Commission amended the title to "Revised request for a scientific opinion on frozen and dried formulations from whole yellow mealworm (*Tenebrio molitor* larva) as a novel food".

## 1.2. Interpretation of the Terms of Reference

Given the proposed intended uses and in accordance to Art 5 of the Commission Implementing Regulation (EU) 2017/2469[1] stating 'where it cannot be excluded that a novel food intended for a particular group of the population would be also consumed by other groups of the population, the safety data provided shall also cover those groups', it was clarified that the target population is the general population.

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

## 1.3. 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, 2021). Following a positive vote of the Standing Committee on Plants, Animals, Food and Feed (Novel Food and Toxicological Safety section) on 03 May 2021, the European Commission adopted on 01 June 2021 the Commission Implementing Regulation (EU) 2021/882[2] authorising the placing on the market of dried yellow mealworm as an NF according to Regulation (EU) 2015/2283.

# 2. Data and methodologies

## 2.1. Data

The safety assessment of this NF is based on data supplied in the application and information submitted by the applicant following EFSA requests for supplementary information. During the assessment, the Panel identified additional data which were not included in the application.

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

[2] 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.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283[3] 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 of the available (proprietary, confidential and published) scientific data (including both data in favour and not in favour) that are pertinent to the safety of the NF.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data requested by the applicant to be protected comprise the detailed description of the production process, analytical data on the composition of the NF, stability studies, intake assessment, proposed use and use levels estimates, protein digestibility study, cytotoxicity study and full study reports.

### 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.

Additional information which was not included in the application was retrieved by literature search following a search strategy and standard operating procedure as described by UCT Prague (2020).

This assessment concerns only the risks that might be associated with consumption of the NF under the proposed conditions of use and is not an assessment of the efficacy of the NF with regard to any claimed benefit.

## 3. Assessment

### 3.1. Introduction

The NF subject of the application is frozen and dried formulations of the *Tenebrio molitor* larva (yellow mealworm), an insect species that belongs to the family of Tenebrionidae (darkling beetles). The NF falls under the category 'food consisting of, isolated from or produced from animals or their parts' as described in Article 3(2)(v) of Regulation (EU) 2015/2283. The NF is produced by farming and processing yellow mealworms. The frozen formulation consists mainly of water, crude protein and fat, whereas the dried formulations consist mainly of crude protein, fat, digestible carbohydrates and fibre. 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 cereal bars, pasta, meat imitates and bakery products. The NF will be added to foods intended for the general population.

### 3.2. Identity of the NF

The NF comprises frozen and freeze-dried formulations of yellow mealworm, whole or in the form of powder. The term 'mealworm' refers to the larval form of *Tenebrio molitor*, an insect species that belongs to the family of Tenebrionidae (darkling beetles). Another identified scientific synonym is *Tenebrio molitor* Linnaeus. 'Yellow mealworms', 'mealworms', 'ver de farine', 'ténébrion meunier' and 'mealworm meal' are some of the common names for *T. molitor* larva or products thereof. The Eastern-Mediterranean region appears to be the area of origin for *T. molitor* sp. (Panagiotakopulu, 2000). However, *T. molitor* is currently present in various regions worldwide, due to colonisation and trade (Panagiotakopulu, 2001). Farmed yellow mealworms are usually fed on wheat flour or bran, although they are omnivorous (Makkar et al., 2014). The NF is intended to be marketed as (a) whole, blanched and frozen *T. molitor* larva (TM frozen), (b) whole, blanched and freeze-dried *T. molitor* larva (TM dried) and (c) as powder of whole, blanched and freeze-dried *T. molitor* larva (TM powder). The entire mealworms are meant for human consumption, no parts are removed. The insects are farmed under controlled rearing conditions.

[3] 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 1852/2001. OJ L 327, 11.12.2015, p. 1–22.

## 3.3. Production process

According to the information provided, the NF is produced in line with Hazard Analysis Critical Control Points (HACCP) principles. The applicant stated that the insects are reared at a facility registered at the Netherlands Food and Consumer Product Safety Authority (NVWA) as food producing company. The production process can be divided into three distinctive parts, i.e. farming, harvesting and post-harvest processing.

The applicant receives the insect from an external breeding facility. Farming includes mating of the adult insect population and rearing of the larvae. The eggs are separated from the adult insects and are hatched separately. After being hatched from the eggs, the light yellow-brown larvae grow in dedicated containers made of certified food contact hard-type plastic (high-density polypropylene). This reduces the probability of plastic ingestion by the larvae (EFSA NDA Panel, 2021). Ingestion of soft-type plastic materials by larvae of the Tenebrionidae family has been reported (Brandon et al., 2018; Yang et al., 2018). The applicant stated that no solvents, antimicrobial substances or veterinary medicinal products are used during the rearing of the larvae.

Yellow mealworms can bioaccumulate chemical agents such as heavy metals, pesticide residues and other undesirable compounds [e.g. polychlorinated biphenyls (PCBs), dioxins] through their feed intake (Bednarska and Świątek, 2016; Ghannem et al., 2018; Houbraken et al., 2016; Lindqvist and Block, 1995; Van der Fels-Klerx et al., 2016; Vijver et al., 2003). The applicant reported that the feed administered to the insects is of plant origin, compliant with Directive 2002/32/EC[4] and produced according to Good Manufacturing Practices (GMP+). Water is provided to the larvae through some components of the feed (e.g. vegetables).

*T. molitor* can be infected by parasites, entomopathogenic fungi and viruses (Gałęcki and Sokół, 2019; Vigneron et al., 2019). For example, this insect species may be infected with tapeworms such as the *Hymenolepis diminuta* ('rat tapeworm') (Shea, 2007) due to the presence of rodents in the farming facilities, as well as with the tapeworm *Hymenolepis nana* and the Newcastle disease virus, all three zoonotic agents which may cause mild symptoms in humans. The applicant stated that measures to control the contamination of the rearing facilities by pests and rodents are in place. The occurrence of parasites in *T. molitor* larvae has been linked with poor hygiene farming conditions in general (Gałęcki and Sokół, 2019). *T. molitor* can be infected by or harbour other viruses such as the invertebrate iridescent virus 29 (IIV-29) (Thomas and Gouranton, 1975; Kelly et al., 1979; Maciel-Vergara and Ros, 2017; Vigneron et al., 2019), and *Acheta domesticus* densovirus (Szelei et al., 2011). However, these viruses are specific to insects, and are not pathogenic for humans or other vertebrates (EFSA Scientific Committee, 2015).

During the rearing of the larvae, deceased insects and faecal contamination are monitored and removed. Mechanical sieving is used to harvest the larvae (~ 7 weeks old), separating them from the substrate, exuvia and faeces. Deceased larvae, which have a darker colour compared to live larvae, are removed after visual inspection. After the harvest, a 12–24 h fasting step is implemented, to allow the larvae to discard their bowel content.

The post-harvesting processing includes the killing of the larvae by freezing, rinsing of the raw frozen larvae with water, blanching (immersion at boiling water with product's temperature > 90°C for at least 2 min). This thermal treatment contributes to the reduction of the microbial load of the larvae as well as to the elimination of potentially present viruses and parasites. Furthermore, this step reduces the activity of enzymes (e.g. tyrosinase/phenoloxidase) (Janssen et al., 2017a) which may induce enzymatic browning in the larvae (Nappi and Vass, 1993; Nappi and Ottaviani, 2000; Sugumaran et al., 2000; Nappi and Christensen, 2005; Vigneron et al., 2014). Subsequently, the blanched larvae are blast chilled (reduction of product's temperature to < 10°C in less than 90 min) and then stored at –18°C (TM frozen). To obtain the 'TM dried' formulation, the frozen larvae are further subjected to freeze-drying (lyophilisation), with the target moisture content being < 5%. The TM powder is obtained via mechanical grinding of the freeze-dried larvae.

TM frozen is stored at –18°C, whereas TM dried and TM powder are stored at 20 ± 5°C (40–50% relative humidity), all in dedicated packaging. For all NF forms, the proposed shelf life is 12 months (further information on the NF's shelf life is provided in Section '3.4.1 Stability').

The Panel considers that the production process is sufficiently described.

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

## 3.4. Compositional data

In order to confirm that the manufacturing process is consistent and adequate to produce on a commercial scale a product with certain characteristics, the applicant provided qualitative and quantitative data on chemical and microbiological parameters for a number of different batches of the NF formulations i.e. (a) TM frozen; (b) TM dried; (c) TM powder. For all parameters, at least five independently produced batches were analysed. Considering the production process, the Panel considers that the two formulations 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. Grinding increases the surface area of the NF and the possibility of cross-contamination, 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 all its constituents cannot be fully identified and/or characterised (EFSA NDA Panel, 2016).

The frozen formulation consists mainly of water, crude protein and fat whereas the freeze-dried formulations of crude protein, fat, digestible carbohydrates and fibre (chitin). The composition of TM dried/TM powder differs from the one of TM frozen due to the reduced water content in the dried formulations (TM dried/powder are concentrates of TM frozen). The results of the proximate analyses of the NF are presented in Table 1. The amino acid, fatty acid, vitamin and mineral composition are reported in Section '3.9 Nutritional information'.

**Table 1:** Batch-to-batch analysis of the NF forms (TM frozen and TM dried)

| Parameter (unit) | Batch number (TM frozen) | | | | | Analytical method |
|---|---|---|---|---|---|---|
| | #1 | #2 | #3 | #4 | #5 | |
| Crude protein (g/100 g of NF) | 14.4 | 14.9 | 16.4 | 16.2 | 15.0 | Kjeldahl (N × 6.25) |
| Fat (g/100 g of NF) | 7.59 | 8.69 | 10.20 | 9.82 | 7.48 | Gravimetric method |
| Digestible carbohydrates (g/100 g of NF) | 1.34 | 1.32 | 1.39 | 1.56 | 1.32 | Titrimetry-Luff School |
| Dietary fibre[(a)] (g/100 g of NF) | 1.2 | 1.2 | 1.2 | 1.5 | 1.5 | Enzymatic-gravimetry-AOAC. 2009.01[(b)] |
| Sugars (g/100 g of NF) | < LOQ[(c)] | < LOQ[(c)] | < LOQ[(c)] | < LOQ[(c)] | 0.6 | HPAEC-PAD[(c)] |
| Ash (g/100 g of NF) | 1.02 | 0.99 | 1.04 | 1.10 | 0.97 | Gravimetric method |
| Moisture (g/100 g of NF) | 74.0 | 72.4 | 70.2 | 69.4 | 74.4 | Gravimetric method |
| Energy value (kJ/100 g of NF) | 560 | 610 | 690 | 680 | 570 | Regulation (EU) 1169/2011[(d)] |
| **Parameter (unit)** | **Batch number (TM dried)** | | | | | **Analytical method** |
| | **#6** | **#7** | **#8** | **#9** | **#10** | |
| Crude protein (g/100 g of NF) | 54.5 | 54.3 | 56.6 | 56.6 | 55.9 | Kjeldahl (N × 6.25) |
| Fat (g/100 g of NF) | 28.7 | 28.4 | 28.4 | 28.6 | 28.9 | Gravimetric method |
| Digestible carbohydrates (g/100 g of NF) | 6.06 | 5.84 | 6.53 | 6.37 | 6.24 | Titrimetry-Luff School |
| Dietary fibre[(a)] (g/100 g of NF) | 4.4 | 4.6 | 5.1 | 5.0 | 4.7 | Enzymatic-gravimetry- AOAC. 2009.01[(b)] |
| Sugars (g/100 g of NF) | < LOQ[(c)] | < LOQ[(c)] | < LOQ[(c)] | < LOQ[(c)] | < LOQ[(c)] | HPAEC-PAD[(c)] |
| Ash (g/100 g of NF) | 3.68 | 3.77 | 4.02 | 4.09 | 3.76 | Gravimetric method (500–550°C) |
| Moisture (g/100 g of NF) | 3.13 | 3.16 | 0.66 | 0.58 | 1.77 | Gravimetric method (102°C) |
| Energy value (kJ/100 g of NF) | 2,100 | 2,100 | 2,200 | 2,200 | 2,200 | Regulation (EU) 1169/2011 |

(a): Chitin is the main form of dietary fibre in the NF.
(b): AOAC: Association of Official Analytical Chemists.
(c): Total sugars were calculated as the sum of individual sugars (excl. galactose), i.e. glucose, fructose, lactose, saccharose, maltose analysed by HPAEC-PAD (High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection). The LOQ for each individual sugar is < 0.1.

(d): 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 Directive 1999/10/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, p. 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 Schlüter, 2013)] and the use of whole insects, the variation of compositional values is acceptable.

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

Chitin is the main form of dietary fibre in *T. molitor* larvae (Finke, 2007; Hahn et al., 2018; Han and Heinonen, 2020). It is a linear polysaccharide constituted by β-(1,4)-linked 2-amino-2-deoxy-β-D-glucopyranose and 2-acetamido-2-deoxy-β-D-glucopyranose residues (Muzzarelli, 1973; Roberts, 1992). The physicochemical nature of chitin is intrinsically related to its source (Kumirska et al., 2011). The applicant provided analytical data on the levels of chitin in 5 independently produced batches in TM dried. The Panel notes that a nationally or internationally recognised reference method for the analytical determination of chitin does not exist. The chitin content in the TM dried was determined 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 in different insects. Based on the lowest and highest chitin values in TM dried and on the dry matter content of the NF formulations, the chitin values in TM frozen fall within the range of ~ 2.0 to 2.6 g/100 g. The Panel considers that the differences between the content of dietary fibre (Table 1) and chitin (Table 2) are due to the different analytical methods utilised.

**Table 2:** Chitin content in the NF (TM dried)

| | Batch number (TM dried) | | | | |
|---|---|---|---|---|---|
| | **#6** | **#7** | **#8** | **#9** | **#10** |
| ADF (g/100 g NF)[(a)] | 7.6 | 7.6 | 8.1 | 8.5 | 8.2 |
| ADL (g/100 g NF)[(b)] | < 1.5 | < 1.5 | < 1.5 | < 1.5 | < 1.5 |
| Chitin (g/100 g NF)[(c)] | 7.6 | 7.6 | 8.1 | 8.5 | 8.2 |

(a): ADF = Acid Detergent Fibre.
(b): ADL = Acid Detergent Lignin, LOQ = 1.5.
(c): Chitin calculated as ADF-ADL. The results may present a small overestimation of chitin due to the uncertainty regarding the ADL numerical value.

Levels of heavy metals in TM dried are reported in Table 3. The applicant compared the values to the maximum levels for other foods as set in Regulation (EC) No 1881/2006[5]. The Panel notes that the levels of heavy metals reported for the NF are comparable to those set for other foods, and that in the current EU legislation, no maximum levels of heavy metals are set for insects as food.

Analytical data on the levels of aflatoxins B1, B2, G1, G2, ochratoxin A, nivalenol, deoxynivalenol, zearalenone, T-2 toxin, HT-2 toxin and, upon EFSA's request, fumonisin B1 and fumonisin B2, were provided (Table 3). Values were lower than the maximum levels set in Regulation (EC) No 1881/2006. The Panel notes that in the current EU legislation no maximum levels of mycotoxins are set for insects as food.

The contents of dioxins and dioxin-like compounds were provided by the applicant (Table 3) and values were lower than the maximum levels for meat and meat products as set in Regulation (EC) No 1881/2006. The Panel notes that in the current EU legislation no maximum levels of dioxins and dioxin-like compounds are set for insects as food.

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

**Table 3:** Heavy metals, mycotoxins and dioxins in TM dried

| Parameter (unit) | Analytical method | Batch number | | | | |
|---|---|---|---|---|---|---|
| | | #6 | #7 | #8 | #9 | #10 |
| **Heavy metals (mg/kg)** | | | | | | |
| Arsenic | Internal method, ICP-MS[(a)] | 0.16 | 0.17 | 0.16 | 0.17 | 0.16 |
| Mercury | | 0.0048 | 0.0045 | 0.0039 | 0.0046 | 0.0041 |
| Lead | | < 0.02 | < 0.01 | < 0.01 | < 0.02 | < 0.01 |
| Cadmium | | 0.081 | 0.076 | 0.076 | 0.082 | 0.075 |
| **Mycotoxins (µg/kg)** | | | | | | |
| Aflatoxins B1 | Internal Method, IAC-LC-FLD[(b)] | < 0.10 | < 0.10 | < 0.10 | < 0.10 | < 0.10 |
| Aflatoxins B2 | | < 0.04 | < 0.04 | < 0.04 | < 0.04 | < 0.04 |
| Aflatoxins G1 | | < 0.10 | < 0.10 | < 0.10 | < 0.10 | < 0.10 |
| Aflatoxins G2 | | < 0.06 | < 0.06 | < 0.06 | < 0.06 | < 0.06 |
| Aflatoxins (Sum of B1, B2, G1, G2) | | < 0.30 | < 0.30 | < 0.30 | < 0.30 | < 0.30 |
| Ochratoxin A | Internal Method, IAC-LC-FLD[(b)] | < 0.4 | < 0.4 | < 0.4 | < 0.4 | < 0.4 |
| Nivalenol | Internal Method, LC-MS/MS[(c)] | < 20 | < 20 | < 20 | < 20 | < 20 |
| Deoxynivalenol | | < 20 | < 20 | < 20 | < 20 | < 20 |
| Zearalenone | | < 10 | < 10 | < 10 | < 10 | < 10 |
| T-2 Toxin | | < 10 | < 10 | < 10 | < 10 | < 10 |
| HT-2 Toxin | | < 10 | < 10 | < 10 | < 10 | < 10 |
| Sum T-2 and HT-2 | | < 20 | < 20 | < 20 | < 20 | < 20 |
| Fumonisin B1 | Internal adaptation of NEN-EN 17194:2017-o, LC-MS/MS[(c)] | < 12 | < 12 | < 12 | < 12 | < 12 |
| Fumonisin B2 | | < 5 | < 5 | < 5 | < 5 | < 5 |
| **Dioxins (pg/g fat)** | | | | | | |
| Sum of dioxins and dl-PCBs (UB, WHO-TEQ$_{2005}$)[(e)] | EC 2017/644, GC-MS/MS[(d)] | 0.290 | 0.285 | 0.315 | 0.312 | 0.318 |

(a): ICP-MS: Inductively Coupled Plasma Mass Spectrometry.
(b): IAC-LC-FLD: immunoaffinity chromatography-liquid chromatography/fluorescence detector.
(c): LC-MS/MS: Liquid Chromatography-tandem Mass Spectrometry.
(d): GC-MS/MS: Gas Chromatography-tandem Mass Spectrometry.
(e): Sum of dioxins and dl-PCBs (WHO-TEQ$_{2005}$): sum of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls by using toxicity equivalence factors (TEF) set by World Health Organization in 2005.

Analytical data of the pesticide levels for five independently produced batches of TM dried have been provided. The results showed that most of the analysed pesticide levels in TM dried are below the limits of detection (LOD) or quantification (LOQ) of the analytical method used (GC-MS ITD Equal CEN/TR 16468). Piperonyl butoxide (PBO) and prosulfocarb have been quantified in some of the analysed NF batches at concentrations lower than the maximum residue levels (MRL) set for other foodstuffs by Codex and by Regulation (EC) No 396/2005[6], accordingly.

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

The applicant provided analytical data for biogenic amines for five independently produced batches of TM dried and TM powder. The range of the reported values in TM dried were 2.7–6.56 mg/kg for histamine, 154–197 mg/kg for spermidine, 22.3–28 mg/kg for spermine and 419–460 mg/kg for putrescine. In TM powder, the respective ranges were 1.75–2.7 mg/kg for histamine, 119–123 mg/kg for spermidine, 168–178 mg/kg for spermine and 230–236 mg/kg for putrescine. No legal maximum limits have been established for spermidine and spermine in foods. Similar concentrations have been reported in legumes (206 mg/kg and 69 mg/kg, respectively), cereals (353 mg/kg and 146 mg/kg, respectively), fresh meat (13 mg/kg and 69 mg/kg, respectively) and cheese (38 mg/kg and 3 mg/kg,

[6] Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. OJ L 70, 16.3.2005, p. 1–16.

respectively) (Muñoz-Esparza et al., 2019). The histamine values were much lower than the limit of 200 mg/kg for histamine in fishery products set in Regulation (EC) No 2073/2005[7]. The Panel notes the relatively high levels of putrescine reported in the NF and that no legal limit has been established for putrescine in any food although it may accumulate at very high concentration in cheese (up to 1,560 mg/kg), fermented sausages (up to 1,550 mg/kg) and fish sauces (up to 1,220 mg/kg) (EFSA BIOHAZ Panel, 2011). Formation of biogenic amines can occur by endogenous biosynthesis, uptake from the feed source and by bacteria of the intestinal microbiota of insects. It can also occur during food processing and storage as result of bacterial contamination (EFSA BIOHAZ Panel, 2011). Upon EFSA's request, the applicant analysed the NF for *Pseudomonas aeruginosa*, which, belonging to the Pseudomonas genus, could have contributed to the occurrence of biogenic amines in the NF. For all forms of the NF, *P. aeruginosa* was reported at levels < 10 cfu/g.

The applicant provided microbiological data on five independently produced batches of all NF forms (TM frozen, TM dried, TM powder) (Table 4). The Panel notes that for the TM frozen and TM dried the applicant did not provide the actual values of the total aerobic count, but instead the quantification limits as defined by the dilutions used upon the analyses. However, the Panel notes that the microbiological values of the analysed samples do not exceed the specification limits.

**Table 4:** Microbiological analyses of the NF

| **Parameter (unit)** | **Batch number (TM frozen)** | | | | | |
|---|---|---|---|---|---|---|
| | **Method** | **#1** | **#2** | **#3** | **#4** | **#5** |
| Total aerobic count (cfu/g) | Equivalent to ISO 4833 | < 1,000 | < 1,000 | < 1,000 | < 1,000 | < 1,000 |
| *Enterobacteriaceae* (cfu/g) | Equivalent to NEN-ISO 21528-2 | < 10 | < 10 | < 10 | < 10 | < 10 |
| *Escherichia coli* (cfu/g) | ISO:16649-2:2001 | < 10 | < 10 | < 10 | < 10 | < 10 |
| *Listeria monocytogenes* (in 25 g) | Equivalent to NEN-EN-ISO 11290-1 | ND | ND | ND | ND | ND |
| *Salmonella spp.* in 25 g) | PCR fast method (equivalent to ISO 6579) | ND | ND | ND | ND | ND |
| *Bacillus cereus* (spores) (cfu/g) | Equivalent to ISO 7932 | < 10 | < 10 | < 10 | < 10 | < 10 |
| Coagulase positive staphylococci (cfu/g) | Equivalent to NEN-EN-ISO 6888-2, 37°C | < 10 | < 10 | < 10 | < 10 | < 10 |
| *Campylobacter* spp. (cfu/g) | NEN-EN-ISO 10272-1 | ND | ND | ND | ND | ND |
| *Clostridium perfringens* (in 25 g) | Equivalent to ISO 7937 | < 10 | < 10 | < 10 | < 10 | < 10 |
| Yeasts and moulds (cfu/g) | Equivalent to ISO 7954:1987 | < 10 | < 10 | < 10 | < 10 | < 10 |
| **Parameter (unit)** | **Batch number (TM dried)** | | | | | |
| | **Method** | **#6** | **#7** | **#8** | **#9** | **#10** |
| Total aerobic count (cfu/g) | Equivalent to ISO 4833 | < 1,000 | < 1,000 | < 1,000 | < 1,000 | < 1,000 |
| *Enterobacteriaceae* (cfu/g) | Equivalent to NEN-ISO 21528-2 | < 10 | < 10 | < 10 | < 10 | < 10 |
| *Escherichia coli* (cfu/g) | ISO:16649-2:2001 | < 10 | < 10 | < 10 | < 10 | < 10 |
| *Listeria monocytogenes* (in 25 g) | Equivalent to NEN-EN-ISO 11290-1 | ND | ND | ND | ND | ND |
| *Salmonella spp.* (in 25 g) | PCR fast method (equivalent to ISO 6579) | ND | ND | ND | ND | ND |
| *Bacillus cereus* (spores) (cfu/g) | Equivalent to ISO 7932 | < 10 | < 10 | < 10 | < 10 | < 10 |
| Coagulase positive staphylococci (cfu/g) | Equivalent to NEN-EN-ISO 6888-2, 37°C | < 10 | < 10 | < 10 | < 10 | < 10 |
| *Campylobacter* spp. (cfu/g) | NEN-EN-ISO 10272-1 | ND | ND | ND | ND | ND |
| *Clostridium perfringens* (in 25 g) | NEN-EN-ISO 10272-1 | < 10 | < 10 | < 10 | < 10 | < 10 |
| Yeasts and moulds (cfu/g) | Equivalent to ISO 7954:1987 | < 10 | < 10 | < 10 | < 10 | < 10 |

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

| Parameter (unit) | Batch number (TM dried) | | | | | |
|---|---|---|---|---|---|---|
| | Method | #11 | #12 | #13 | #14 | #15 |
| Total aerobic count (cfu/g) | Equivalent to ISO 4833 | < 10 | < 40 | < 10 | < 10 | < 10 |
| *Enterobacteriaceae* (cfu/g) | Equivalent to NEN-ISO 21528-2 | < 10 | < 10 | < 10 | < 10 | < 10 |
| *Escherichia coli* (cfu/g) | ISO:16649-2:2001 | < 10 | < 10 | < 10 | < 10 | < 10 |
| *Listeria monocytogenes* (in 25 g) | Equivalent to NEN-EN-ISO 11290-1 | ND | ND | ND | ND | ND |
| *Salmonella spp.* (in 25 g) | PCR fast method (equivalent to ISO 6579) | ND | ND | ND | ND | ND |
| *Bacillus cereus* (spores) (cfu/g) | Equivalent to ISO 7932 | < 10 | < 10 | < 10 | < 10 | < 10 |
| Coagulase positive staphylococci (cfu/g) | Equivalent to NEN-EN-ISO 6888-2, 37°C | < 10 | < 10 | < 10 | < 10 | < 10 |
| *Campylobacter* spp. (cfu/g) | NEN-EN-ISO 10272-1 | ND | ND | ND | ND | ND |
| *Clostridium perfringens* (in 25 g) | NEN-EN-ISO 7937 | < 10 | < 10 | < 10 | < 10 | < 10 |
| Yeasts and moulds (cfu/g) | Equivalent to ISO 7954:1987 | < 10 | < 10 | < 10 | < 10 | < 10 |

ND: not detected; cfu: colony forming units.

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

### 3.4.1. Stability

The applicant provided data on the microbiological profile of five batches of 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 12 months. Microbiological data at 3, 6 and 9 months were also provided for TM frozen and TM dried, falling within the range of the values reported for month 0 and for month 12. Microbiological data at 6 months were provided for TM powder, falling within 0–12 months values. The Panel notes that the microbiological values do not exceed the given specification limits.

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

| Parameter (unit) | Batch number (TM frozen) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | #1 | #2 | #3 | #4 | #5 | #16 | #17 | #18 | #19 | #20 |
| Time (months) | 0 months | | | | | 12 months | | | | |
| Total aerobic count (cfu/g) | < 1,000 | < 1,000 | < 1,000 | < 1,000 | < 1,000 | 2,100 | < 4,000 | < 4,000 | < 1,000 | < 4,000 |
| *Enterobacteriaceae* (cfu/g) | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 |
| *Escherichia coli* (cfu/g) | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 |
| *Listeria monocytogenes (in 25 g)* | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| *Salmonella* spp. (in 25 g) | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| *Bacillus cereus* (cfu/g) | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 |
| Coagulase positive staphylococci (cfu/g) | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 |
| *Clostridium perfringens* (cfu/g) | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 |
| Yeasts and moulds (cfu/g) | < 40 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 |
| Campylobacter (in 25 g) | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| | Batch number (TM dried) | | | | | | | | | |
| | #6 | #7 | #8# | #9 | #10 | #21 | #22 | #23 | #24 | #25 |
| Time (months) | 0 months | | | | | 12 months | | | | |
| Total aerobic count (cfu/g) | < 1,000 | < 1,000 | < 1,000 | < 1,000 | < 1,000 | < 10 | < 10 | < 10 | < 10 | < 10 |
| *Enterobacteriaceae* (cfu/g) | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 |
| *Escherichia coli* (cfu/g) | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Listeria monocytogenes* (in 25 g) | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| *Salmonella* spp. (in 25 g) | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| *Bacillus cereus* (cfu/g) | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 |
| Coagulase positive staphylococci (cfu/g) | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 |
| *Clostridium perfringens* (cfu/g) | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 |
| Yeasts and moulds (cfu/g) | < 40 | < 10 | < 10 | < 10 | < 10 | < 40 | < 10 | < 10 | < 10 | < 10 |
| | **Batch number (TM powder)** | | | | | | | | | |
| | **#11** | **#12** | **#13** | **#14** | **#15** | **#26** | **#27** | **#28** | **#29** | **#30** |
| Time (months) | **0 months** | | | | | **12 months** | | | | |
| Total aerobic count (cfu/g) | < 10 | < 40 | < 40 | < 10 | < 40 | < 40 | 13,000 | < 10 | < 10 | < 40 |
| *Enterobacteriaceae* (cfu/g) | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 |
| *Escherichia coli* (cfu/g) | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 |
| *Listeria monocytogenes (in 25 g)* | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| *Salmonella* spp. (in 25 g) | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| *Bacillus cereus* | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 |
| Coagulase positive staphylococci (cfu/g) | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 |
| *Clostridium perfringens* (cfu/g) | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 |
| Yeasts and moulds (cfu/g) | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 |

ND: not detected; cfu: colony forming units.

Following EFSA's request, the applicant provided analytical data on the water activity and the oxidative status of the fats in the NF (TM powder) (Table 6). The peroxide values (PV), p-anisidine (PA) values and free fatty acids (FFA) percentages were determined for a period covering the proposed shelf life (12 months). The Panel noted the high variability among the p-anisidine values. The applicant informed that the five batches of the NF analysed at t = 12 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 6:** Water activity and oxidative status of fat in TM powder during the proposed shelf life

| **Parameter (unit)** | **Analytical method** | **Batch number (TM powder)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **0 months** | | | | | **12 months** | | | | |
| | | **#11** | **#12** | **#13** | **#14** | **#15** | **#26** | **#27** | **#28** | **#29** | **#30** |
| **aw**[(a)] | NEN-EN-ISO 18787:2017 | 0.19 | 0.17 | 0.18 | 0.2 | 0.17 | 0.2 | 0.19 | 0.19 | 0.18 | 0.19 |
| **FFA**[(b)] (expressed as % oleic acid of total fat) | NEN-EN-ISO 660:2009 | 1.1 | 1.7 | 0.8 | 1.9 | 1.7 | 1.5 | 1.7 | 1.8 | 1.9 | 1.8 |
| **PV**[(c)] (meq $O_2$/kg fat[(d)]) | NEN-EN-ISO 3960:2010 | 1.8 | 1.7 | 0.7 | 1.9 | 2.4 | 1.2 | 2.2 | 1.9 | 2.6 | 3.2 |
| **p-Anisidine value** | NEN-EN-ISO 6885:2000 | 1.2 | 2.4 | 7.7 | 2.9 | 3 | 1 | 3.7 | 6.6 | 4.9 | 3.9 |

(a): Water activity.
(b): Free fatty acids.
(c): Peroxide value.
(d): Meq: milliequivalents.

### Stability in the intended for use 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 Proposed uses and use levels). The applicant investigated the lipid hydrolysis and oxidation, and the microbiological profile of products containing the NF as an ingredient (TM patties, TM snacks, TM biscuits) after manufacture and after different duration of storage. In addition, the applicant reported the concentration of polycyclic aromatic hydrocarbons (PAHs) (TM snack), furan (TM patties, TM-biscuits), free 3-MCPD (TM patties, TM-biscuits) and acrylamide (TM-biscuits) after manufacture of the products.

The TM patties (5 samples) comprised 45% of TM frozen and a mixture of vegetables, cereals, eggs and spices. The TM patties were pre-cooked in sunflower oil (140°C for t < 1 min) before packaging and freezing (t < –4°C), with an intended shelf-life of 6 months. The reported water activity ranged from 0.95 to 0.97 during the shelf life. The average FFA levels in TM patties - expressed as % oleic acid in total fat - were reported in the range of 0.54% and 1.1% at t = 0 and t = 6 months of storage, respectively. Average PV of TM patties were reported to be 4.18 and 5.30 meq/kg fat at t = 0 and t = 6 months, respectively. The p-anisidine value in the products ranged from 34.6 to 67.2 at t = 0 and from 17.5 to 51.8 at t = 6 months. This indicates presence of secondary oxidation products at t = 0. The applicant hypothesised that this is due to frying the TM patty at high temperature in sunflower oil during production. The Panel notes that the microbiological values in TM patty do not raise safety concerns. The applicant analysed further furan and free 3-MCPD (3-monochloropropane-1,2-diol or 3-chloropropane-1,2-diol) at t = 0, and reported that, on average, the furan concentrations were < 5 μg/kg and the free 3-MCPD concentrations < 10 μg/kg in TM patties.

Regarding 'TM snack', i.e. TM dried (22%) mixed with sunflower and pumpkin seeds, thermally processed (roasted at 40°C for 8–10 min) and flavoured, the applicant investigated the shelf life in 5 samples for a period of 6 months. TM snack had an average water activity of 0.42 and 0.45, at t = 0 and t = 6 months, respectively. The average value of FFA was 2.8 and 1.8% of total fat expressed as oleic acid at t = 0 and t = 6 months, respectively. The average PV of the product was 2.02 and 2.12 meq/kg fat at t = 0 and t = 6 months of storage, respectively. The average p-anisidine value in the TM snack ranged from 1.0 to 8.9 at t = 0 and from 1.0 to 3.0 at t = 6 months. The Panel notes that the microbiological values in TM snack do not raise safety concerns. The average PAH4 (benzo[a] anthracene, chrysene, benzo[b]fluoranthene, benzo[a]pyrene) content in the TM snack was below 2 μg/kg at t = 0, much lower than the MRL for PAH4 in different food products other than baby food and food for special medical purposes [Regulation (EC) No 1881/2006].

TM biscuits with 8% TM powder (five samples) baked (at 200°C, 10–12 min) were stored at ambient temperature for 9 months. The water activity in TM biscuits ranged from 0.11 to 0.28. The average value of FFA in TM biscuits was found between 0.54 and 0.40% of total fat expressed as oleic acid at t = 0 and t = 6 months, respectively. The average PV of the product was found between 1.38 and 2.60 meq/kg fat for t = 0 and t = 9 months, respectively. The average p-anisidine value was reported as 4.94 at t = 0 and 4.26 at t = 9 months. The Panel notes that the microbiological values reported in TM biscuits do not raise safety concerns. Three processing contaminants were determined in TM biscuits: furan, free 3-MCPD and acrylamide. The applicant reported average furan levels of 30 μg/kg, and acrylamide of 252 μg/kg. In four samples, free 3-MCPD was found < 10 μg/kg, and in one sample 216 μg/kg.

The Panel notes that the analytical data regarding processing - generated contaminants due to the use of NF as an ingredient in the intended-for-use matrices is limited, and no conclusion can be drawn due to either the absence of proper control samples (ambiguous recipe of control samples) and/or insufficient number of control samples analysed.

The Panel further notes that the food items containing the NF have to comply with existing legislative limits, such as microbiological levels set in Regulation (EC) 2073/2005 and the benchmark levels of acrylamide in bakery products established by Regulation (EU) No 2017/2158[8].

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

[8] 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, p. 24–44.

## 3.5. Specifications

The specifications of the NF are indicated in Table 7.

**Table 7:** Specifications of the NF

| **Description:**<br>TM frozen: whole, blanched and frozen *T. molitor* larva<br>TM dried: whole, blanched and freeze-dried *T. molitor* larva<br>TM powder: whole, blanched, freeze-dried and ground *T. molitor* larva (powder) | | | | |
|---|---|---|---|---|
| **Parameters** | **Unit** | **TM frozen** | **TM dried** | **TM powder** |
| **Moisture** | % w/w | 69–75 | ≤ 5 | ≤ 5 |
| **Crude protein (N × 6.25)** | % w/w | 14–19 | 54–60 | 54–60 |
| **Fat** | % w/w | 7–12.5 | 27–30 | 27–30 |
| of which saturated | % fat | 20–29 | 20–29 | 20–29 |
| **Digestible carbohydrates** | % w/w | 1–2 | 4–8 | 4–8 |
| **Dietary fibre** | % w/w | 1.5–3.5 | 4–6 | 4–6 |
| **Chitin*** | % w/w | ≤ 3 | ≤ 9 | ≤ 9 |
| **Peroxide value** | meq $O_2$/kg fat | ≤ 5 | ≤ 5 | ≤ 5 |
| **Heavy metals** | | | | |
| Lead | mg/kg | ≤ 0.01 | ≤ 0.01 | ≤ 0.01 |
| Cadmium | mg/kg | ≤ 0.05 | ≤ 0.1 | ≤ 0.1 |
| **Mycotoxins** | | | | |
| Aflatoxins (Sum of B1, B2, G1, G2) | µg/kg | ≤ 1 | ≤ 1 | ≤ 1 |
| Deoxynivalenol | µg/kg | ≤ 20 | ≤ 20 | ≤ 20 |
| Ochratoxin A | µg/kg | ≤ 0.1 | ≤ 0.1 | ≤ 0.1 |
| **Sum of dioxins and dl-PCBs (UB, WHO-$TEQ_{2005}$)**** | pg/g fat | ≤ 0.75 | ≤ 0.75 | ≤ 0.75 |
| **Microbiological** | | | | |
| Total aerobic colony count | cfu/g | ≤ $10^5$ | ≤ $10^5$ | ≤ $10^5$ |
| *Enterobacteriaceae* (presumptive) | cfu/g | ≤ 100 | ≤ 100 | ≤ 100 |
| *Escherichia coli* | cfu/g | ≤ 50 | ≤ 50 | ≤ 50 |
| *Listeria monocytogenes* | in 25 g | Not detected | Not detected | Not detected |
| *Salmonella* spp. | In 25 g | Not detected | Not detected | Not detected |
| *Bacillus cereus* (presumptive) | cfu/g | ≤ 100 | ≤ 100 | ≤ 100 |
| Coagulase-positive staphylococci | cfu/g | ≤ 100 | ≤ 100 | ≤ 100 |
| *Sulfite-reducing anaerobes* | cfu/g | ≤ 10 | ≤ 10 | ≤ 10 |
| Yeasts and Moulds | cfu/g | ≤ 100 | ≤ 100 | ≤ 100 |

cfu: colony forming units; UB: Upper Bound; WHO PCDD, PCDF, PCB: sum of polychlorinated dibenzo-para-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls expressed as World Health Organization toxic equivalent.

*: 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).

**: Sum of dioxins and dl-PCBs (WHO-$TEQ_{2005}$): sum of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls by using toxicity equivalence factors (TEF) set by World Health Organization in 2005.

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

'Yellow mealworms are consumed as part of the customary diet or for medicinal purposes in some non-EU countries worldwide. Their consumption by humans has been reported in Thailand (Hanboonsong et al., 2013), China (Feng et al., 2018) and Mexico (Ramos-Elorduy, 1997, 2009; Ramos-Elorduy and Moreno, 2004). Yellow mealworms are among the insect species permitted to be consumed as food in Korea by the Korean Food and Drug Administration (KFDA) (Kim et al., 2017). Additionally, in Australia and New Zealand yellow mealworms are considered as non-traditional, not novel foodstuff (FSANZ, 2020). Since 1 May 2017, *T. molitor* larva is among the insect species that can

be legally introduced in the Swiss market as food (whole, chopped or ground)' (EFSA NDA Panel, 2021).

## 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 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 forms (TM frozen, TM dried and TM powder) are proposed to be used as ingredients in several food products. The food categories defined using the FoodEx2 hierarchy (EFSA, 2015) and the maximum use levels are reported in Table 8. The applicant intends to use the different NF forms singularly in the respective food categories, and not in combination.

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

| FoodEx2 Level | FoodEx2 Code | Food category | Maximum use levels (g NF/100 g) | | |
|---|---|---|---|---|---|
| | | | TM dried | TM powder | TM frozen |
| 4 | A005L | Multigrain bread and rolls | 10 | 10 | 30 |
| 3 | A005Y | Crackers and breadsticks | 10 | 10 | 30 |
| 3 | A00EY | Cereal bars | 15 | 15 | 30 |
| 4 | A009X | Biscuits, sweet, plain | 8 | 8 | 30 |
| 5 | A007L | Dried pasta | 5 | 5 | 15 |
| 5 | A007Y | Dried stuffed pasta | 15 | 15 | 30 |
| 4 | A0CSK | Pre-mixes (dry) for baked products | 15 | 15 | 30 |
| 5 | A045N | Tartar sauce | 10 | 10 | 30 |
| 4 | A03VD | Potato based dishes | 5 | 5 | 15 |
| 4 | A03VM | Legumes based dishes | 5 | 5 | 15 |
| 4 | A03ZN | Pizza and pizza-like dishes | 5 | 5 | 15 |
| 4 | A040N | Pasta based dishes, cooked | 5 | 5 | 15 |
| 4 | A02PN | Whey powder | 20 | 20 | 40 |
| 3 | A03TE | Meat imitates | 50 | 50 | 80 |
| 2 | A041K | Soups and salads | 5 | 5 | 20 |
| 4 | A0EQX | Chips/crisps | 20 | 20 | 40 |
| 2 | A03MA | Beer and beer-like beverages | 1 | 1 | 1 |
| 2 | A03PM | Mixed alcoholic drinks | 1 | 1 | 1 |
| 2 | A04QF | Unsweetened spirits and liquors | 1 | 1 | 1 |
| 4 | A0EQD | Chocolate and similar | 10 | 10 | 30 |
| 3 | A06HL | Snacks other than chips and similar | 100 | 100 | 100 |
| 3 | A01BJ | Primary derivatives from nuts and similar seeds | 30 | 30 | 40 |
| 5 | A0BAV | Chickpeas (without pods) | 30 | 30 | 40 |
| 3 | A014C | Tree nuts | 30 | 30 | 40 |
| 3 | A015F | Oilseeds | 30 | 30 | 40 |
| 4 | A02QC | Frozen yoghurt | 5 | 5 | 15 |
| 5 | A03XG | Meat balls | 16 | 16 | 40 |
| 5 | A03XF | Meat burger | 16 | 16 | 40 |

### 3.7.3. Anticipated intake of the NF

EFSA performed an intake assessment of the anticipated daily intake of the NF based on the applicant's proposed uses and maximum proposed use levels (Table 8), using individual data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011). Since the applicant intends to use the different NF forms singularly in the respective food categories and not in combination, for the calculation of the anticipated daily intake the scenario that leads to the inclusion of the highest amount of NF dry matter in each food category was used. The lowest and highest mean and 95th percentile anticipated daily intake of the NF (on a mg/kg body weight (bw) basis), among the EU dietary surveys, are presented in Table 9.

The estimated daily intake of the NF for each population group from each EU dietary survey is available in the excel file annexed to this scientific opinion (under supporting information).

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

| Population group | Age (years) | Mean intake (mg/kg bw per day) | | P95th intake (mg/kg bw per day) | |
|---|---|---|---|---|---|
| | | Lowest[(a)] | Highest[(a)] | Lowest[(b)] | Highest[(b)] |
| Infants | < 1 | 9 | 179 | 50 | 731 |
| Young children[(d)] | 1–< 3 | 81 | 651 | 286 | 1,257 |
| Other children | 3–< 10 | 71 | 580 | 242 | 1,239 |
| Adolescents | 10–< 18 | 41 | 248 | 145 | 707 |
| Adults[(c)] | ≥ 18 | 34 | 359 | 107 | 580 |

(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 26/5/2021. The lowest and the highest averages observed among all EU surveys are reported in these columns.
(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 26/5/2021. The lowest and the highest P95th observed among all EU surveys are reported in these columns (P95th based on less than 60 individuals are not considered).
(c): Includes elderly and very elderly persons, and pregnant and lactating women.
(d): Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).

### 3.7.4. Estimate of exposure to undesirable substances

Based on the highest P95th intake estimate (Table 9), EFSA estimated exposure to undesirable substances (heavy metals, toxins) from the NF for all population groups. The specification limits (Table 7) were used as maximum concentrations of the undesirable substances. When specification limits for a substance of possible concern have not been proposed, the maximum values reported for the analysed batches were used. The Panel considers that consumption of the NF under the proposed uses and use levels does not contribute substantially to the overall exposure to the analysed undesirable substances through diet.

## 3.8. 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. TM dried and TM powder consist mainly of protein, fat, digestible carbohydrates, dietary fibre (mainly chitin) and inorganic matter. In comparison to TM dried and TM powder, the composition of TM frozen differs due to its high water content. The energy value of TM frozen is on average 620 kJ/100 g and of TM dried/TM powder 2,160 kJ/100 g (Table 1). Analytical data on the amino acid composition, the fatty acid content, minerals and vitamins have been provided for five batches of TM powder.

The NF contains on average 15.4 (± 0.9) g crude protein per 100 g TM frozen, 55.6 (± 1.1) g crude protein per 100 g TM dried/TM powder, calculated using a protein-conversion-factor of 6.25. The Panel notes that the use of this conventional factor overestimates the level of true protein content in the yellow mealworm due to the presence of considerable amounts of non-protein nitrogen derived mainly from chitin (Janssen et al., 2017b). Based on the amino acid profile of the insects, Janssen et al. (2017b) proposed a conversion factor of 4.76 for yellow mealworm. Using this factor, the protein

content of the NF amounts to 11.7 g/100 g in TM frozen and 42.3 g/100 g in TM dried/TM powder. For regulatory purposes for nutritional labelling, protein is defined as the total nitrogen measured by the Kjeldahl method multiplied by a nitrogen-to-protein conversion factor of 6.25 [Regulation (EU) No 1169/2011 on the provision of food information to consumers].

The applicant quantified the amino acids in five batches of the TM dried according to ISO 13903:2005 and Commission Regulation (EC) No 152/2009[9] (Appendix A), and all essential amino acids were found to be present, including the sulfur-containing ones. In TM frozen, the amino acid content is higher than that of raw rice (brown or husked), close to those of pork (edible flesh) and whole grain wheat and lower than those of beef (edible flesh) and chicken (edible flesh). Regarding TM dried/TM powder, the content of all individual amino acids and the total amino acid content are higher than those of the foods used for comparison (Appendix B).

In addition, the applicant conducted a study of the true ileal protein digestibility during transit through the dynamic *in vitro* gastrointestinal model (tiny-TIM). Casein was used as a reference protein. The tests were conducted by an accredited laboratory in accordance with GLP. The nitrogen digestibility was expressed as percentage of the total nitrogen intake, including non-protein nitrogen. The true ileal digestibility was found to be higher for casein (75.3 ± 1.4%) compared to TM dried (64.0 ± 0.0%), indicating that proteins of TM dried are less bio-accessible than casein. Following the recommendation by FAO (2013), the protein quality was determined by the 'Digestible Indispensable Amino Acid Score (DIAAS)'. The DIAAS score for TM dried corresponded to 51%, compared to casein with DIAAS score of 91%. The limiting amino acids, both for casein and TM dried, were the sulfur-containing amino acids.

If the NF entirely replaces other protein sources of higher quality, it may negatively impact protein nutrition if the overall protein intake is low. Based on the high (95$^{th}$ percentile) intake levels of the NF (Section 3.7.3, Table 9) with a maximum content of protein of ~ 60% (TM dried/TM powder) (Section 3.5, Table 7), the corresponding protein intake per kg bw per day from the NF would amount to 0.44 g for infants, 0.75 g for young children, 0.74 g for other children, 0.42 g for adolescents and 0.35 g for adults. These intakes would correspond to up to 33%, 78%, 87%, 47% and 42% of respective dietary reference values (PRIs) (EFSA NDA Panel, 2012) for protein for infants, young children, other children, adolescents and adults. Taking into account that the NF will not be the sole source of dietary protein is integrated into a varied and mixed diet, and that the average protein intake in the EU population is high and frequently above DRVs (EFSA NDA Panel, 2012), the consumption of the NF should not negatively impact protein nutrition.

The major fatty acids in the NF are oleic acid, linoleic acid and palmitic acid (Appendix C). On average, saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids constitute 24.8%, 48.8% and 26.4% of the total fatty acids, respectively (ISO 12966-2/4). The average *trans* fatty acid content is 0.78% of total fatty acids.

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

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

**Table 10:** Content of micronutrients (minerals and vitamins) in the TM dried

| Parameter (unit) | Analytical method | #6 | #7 | #8 | #9 | #10 |
|---|---|---|---|---|---|---|
| **Minerals** | | | | | | |
| Calcium (mg/100 g) | ICP-MS | 75 | 78 | 80 | 80 | 78 |
| Copper (mg/100 g) | | 1.5 | 1.4 | 1.5 | 1.6 | 1.5 |
| Iron (mg/100 g) | | 5.3 | 5.5 | 5.2 | 5.5 | 5.6 |
| Magnesium (mg/100 g) | | 200 | 190 | 190 | 200 | 190 |
| Manganese (mg/100 mg) | | 0.69 | 0.69 | 0.64 | 0.65 | 0.67 |
| Phosphorus (mg/100 g) | | 740 | 800 | 840 | 800 | 830 |
| Potassium (mg/100 g) | | 1,000 | 1,000 | 1,100 | 1,100 | 1,100 |
| Sodium (mg/100 g) | | 190 | 190 | 210 | 220 | 200 |

[9] 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, p. 1–130.

| Parameter (unit) | Analytical method | #6 | #7 | #8 | #9 | #10 |
|---|---|---|---|---|---|---|
| Zinc (mg/100 g) | | 14 | 14 | 15 | 16 | 14 |
| Iodine (μg/100 g) | | 0.051 | 0.051 | 0.049 | 0.047 | 0.047 |
| Selenium (μg/100 g) | | 0.055 | 0.029 | 0.036 | 0.040 | 0.036 |
| Boron (mg/100 g) | ICP-OES | 0.39 | 0.35 | 0.30 | 0.35 | 0.40 |
| Molybdenum (mg/100 g) | | < 0.2 | < 0.2 | < 0.2 | < 0.2 | < 0.2 |
| **Vitamins** | | | | | | |
| Retinol (μg/100 g) | EN 12823-1 2014 | < 21 (LOQ) | < 21 (LOQ) | < 21 (LOQ) | < 21 (LOQ) | < 21 (LOQ) |
| Thiamin (mg/100 g) | EN 14122:2003, mod. | 0.30 | 0.31 | 0.33 | 0.33 | 0.33 |
| Riboflavin (mg/100 g) | EN 14152:2003, mod. | 0.75 | 0.79 | 0.76 | 0.72 | 0.7 |
| Niacin (mg/100 g) | EN 15652:2009 | 1.12 | 1.09 | 1.17 | 1.15 | 1.13 |
| Pantothenic acid (mg/100 g) | AOAC 2012.16 | 5.31 | 5.44 | 6.34 | 6.24 | 5.75 |
| Pyridoxine hydrochloride (mg/100 g) | EN 14164 | 0.180 | 0173 | 0.181 | 0.307 | 0.194 |
| Biotin (μg/100 g) | LST AB 266.1, 1995 | 172 | 167 | 157 | 192 | 177 |
| Folic acid (μg/100 g) | AOAC 2013.13 | < 5 (LOQ) | < 5 (LOQ) | < 5 (LOQ) | < 5 (LOQ) | < 5 (LOQ) |
| Cyanocobalamin (μg/100 g) | AOAC 2008, vol91 no4 | 0.319 | 0.316 | 0.317 | 0.338 | 0.329 |
| Cholecalciferol (μg/100 g) | EN 12821:2009 | < 0.25 (LOQ) | < 0.25 (LOQ) | < 0.25 (LOQ) | 0.524 | 0.499 |
| Alpha-tocopherol (mg/100 g) | EN 12822:2014 | 1.05 | 1.10 | 1.33 | 1.76 | 1.13 |

LOQ: limit of quantification; ICP-MS: Inductively coupled plasma mass spectrometry; ICP-OES: Inductively coupled plasma-atomic emission spectroscopy; AOAC: Association of Official Analytical Chemists.

It has been reported that chitin can be partially digested in the human stomach by the acidic mammalian chitinase (AMCase) (Paoletti et al., 2009; Muzzarelli et al., 2012). However, Paoletti et al. (2009) suggested that reduction of chitin intake in Western diets may have led to reduced expression of chitinase genes, thus resulting to the loss of catalytic efficacy. The NF contains on average 4.3 g chitin in 100 g of TM frozen and 6.5 g of chitin in 100 g of TM dried/TM powder. The Panel considers that chitin is an insoluble fibre that is not expected to be digested in the small intestine of humans to any significant degree. It is also rather resistant to microbial fermentation and therefore assumed to be excreted mainly unchanged. Additionally, the Panel notes that chitin can bind bivalent minerals (Franco et al., 2004; Anastopoulos et al., 2017) possibly affecting their bioavailability, as reported for dietary fibres in general (Baye et al., 2017).

Insects may contain antinutritional factors (ANFs) such as tannins, oxalates, phytates, hydrogen cyanide (Shantibala et al., 2014; Meyer-Rochow et al., 2021), thiaminases (Nishimune et al., 2000) and protease inhibitors (Eguchi, 1993). The applicant determined the concentrations of total polyphenols, tannins, oxalic acid, phytic acid, hydrogen cyanide and trypsin inhibitors in five independently produced batches of TM dried (Table 10). The reported values in the NF are comparable to the occurrence levels of these compounds in other foodstuffs (Rao and Prabhavathi, 1982; Gupta, 1987; Holmes and Kennedy, 2000; Schlemmer et al., 2009; EFSA CONTAM Panel, 2019).

**Table 11:** Batch-to-batch analysis of antinutritional factors in TM dried

| Parameter (unit) | Analytical method | #6 | #7 | #8 | #9 | #10 |
|---|---|---|---|---|---|---|
| **Total polyphenols** (%) | Folin-Ciocalteu | 0.97 | 0.95 | 1.11 | 1.06 | 1.13 |
| **Tannins** (%) | Folin Denis method | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 |
| **Oxalic acid** (mg/kg) | HPLC/UV, in house method | < 100 | < 100 | < 100 | < 100 | < 100 |
| **Phytic acid** (g/kg) | ANAL-10445 | 2.2 | 2.5 | 2.5 | 2.2 | 2.0 |
| **Hydrogen cyanide** (mg/kg) | NEN-EN 16160:2012 | < 1.5 | < 1.5 | < 1.5 | < 1.5 | < 1.5 |
| **Trypsin inhibition activity** (mg/g) | NEN-EN-ISO 14902 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 |

HPLC/UV: High Performance Liquid Chromatography/Ultraviolet detector.
The Panel considers that taking into account the composition of the NF and the proposed conditions of use, consumption of the NF is not nutritionally disadvantageous.

## 3.10. Toxicological information

No toxicological studies with the NFs were provided by the applicant, apart from cytotoxicity tests with extracts of the NF. The toxicological profile of *T. molitor* larvae has been previously assessed by the Panel (EFSA NDA Panel, 2021). 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 (Ladisch et al., 1967; Attygalle et al., 1991; Brown et al., 1992). 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., 2021).

As reviewed by Komi et al. (2018), chitin has been shown to activate a variety of innate (eosinophils, macrophages) and adaptive immune cells (IL-4/IL-13 expressing T helper type-2 lymphocytes) and this implies the potential to promote hypersensitivity. EFSA identified an article (Niho et al., 1999) (Japanese language, only abstract available in English) stating that no toxic effects related to chitin were observed in F344 rats at concentrations up to 5% of chitin in the diet for 13 weeks (equivalent to 4,500 mg/kg bw per day[10]). No firm conclusions could be drawn by the Panel since only the abstract was accessible (EFSA NDA Panel, 2021).

### 3.10.1. Cytotoxicity

A cytotoxicity assay was provided by the applicant, testing aqueous NF extract (TM powder) on three mammalian cell types (human promyelocytic leukaemia cells, human HeLa cells, Caco-2 cells). Cell survival was quantified by a colorimetric test to measure mitochondrial activity in viable cells. No cytotoxic effect *in vitro* towards mammalian cells was observed at any concentration of NF extract used in the studies up to 250 μg/mL.

### 3.10.2. Summary of toxicological information

No toxicological studies with the NF as testing material were provided, apart from an *in vitro* cytotoxicity study with an aqueous extract of the NF, which did not show any cytotoxic effect towards mammalian cells. No adverse effects were observed in the toxicological studies available in the literature on freeze-dried yellow mealworms.

### 3.10.3. Human data

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

## 3.11. Allergenicity

The Panel has previously considered that the consumption of the NF source (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 and dust mites 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. This may include allergens listed in the Annex II of Regulation (EU) No 1169/2011 (EFSA NDA Panel, 2021).

# 4. Discussion

The NF which is the subject of the application comprises the frozen and dried formulations of the yellow mealworm (*Tenebrio molitor* larva), whole or in the form of powder. The production process is sufficiently described and does not raise safety concerns. The Panel considers that the NF is sufficiently characterised. The composition of TM dried/TM powder differs from the one of TM frozen due to the reduced water content in the dried formulations (TM dried/powder are concentrates of TM frozen). The frozen formulation of the NF consists mainly of water, protein and fat, whereas the dried

[10] Applying a conversion factor of 0.09 (EFSA Scientific Committee, 2012).

formulations consist mainly of protein, fat, digestible carbohydrates and dietary fibre (mainly chitin). The concentrations of contaminants depend mainly on the occurrence of these substances in the insect feed. Provided that the respective EU legislation regarding feed is followed, the consumption of the NF does not raise safety concerns. The Panel notes that there are no safety concerns regarding stability if the NF complies with the proposed specification limits during its entire shelf life.

The applicant intends to market the NF as an ingredient in several food products. The target population is the general population. 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 286 to 1,257 mg NF/kg bw per day at the 95th percentile of the intake distribution. The Panel notes that consumption of the NF under the proposed uses and use levels does not contribute substantially to the total dietary exposure of the population to the analysed undesirable substances (heavy metals, mycotoxins).

The Panel notes that TM dried and TM powder have a high protein content, although the true protein levels in the NF are overestimated due to the presence of non-protein nitrogen of chitin when using the conversion factor of 6.25. The true ileal nitrogen digestibility of the NF (TM dried) is 64.0 ± 0.0%, with a DIAAS value of 51% as compared to casein. The limiting amino acids were the sulfur-containing ones. Considering that the NF will not be the sole source of dietary protein and is integrated into a varied and mixed diet, the consumption of the NF is not expected to negatively impact protein nutrition.

None of the existing upper levels for the analysed micronutrients are exceeded considering the proposed uses and use levels. The reported values for the levels of antinutritional factors in the NF are comparable to those in other foodstuffs. The Panel considers that the main type of fibre in the NF, chitin, is an insoluble fibre not expected to be digested in the small intestine of humans to any significant degree and is assumed to be excreted mainly unchanged. Additionally, the Panel notes that chitin, like other fibres, can possibly reduce the bioavailability of minerals. Taking into account the composition of the NF and the proposed conditions of use, the Panel concludes that the consumption of the NF is not nutritionally disadvantageous. As no adverse effects were observed neither in the toxicological studies available in the literature on freeze-dried yellow mealworms nor were identified from the history of use of the NF and its source, the Panel considers that there are no safety concerns, provided the larvae are reared separately from the adults.

The Panel considers that the consumption of the NF may induce primary sensitisation and allergic reactions to yellow mealworm proteins and may cause allergic reactions in subjects with allergy to crustaceans and dust mites due to cross-reactivity. Additionally, the Panel notes that allergens from the feed (e.g. gluten) may end up in the NF.

# 5. Conclusions

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

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

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the detailed description of the production process, analytical data on the composition of the NF, stability studies, protein digestibility study, cytotoxicity study and full study reports claimed as proprietary by the applicant.

# 6. Recommendation

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

## 7. Steps taken by EFSA

1) On 09 September 2019 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of whole and ground mealworms (*Tenebrio molitor*) larvae as a novel food. Ref. Ares (2019)5170990 - 09/08/2019.
2) On 09 September 2019, a valid application on whole and ground mealworms (*Tenebrio molitor*) larvae, which was submitted by Fair Insects BV (A Protix Company), was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2018/0802) and the scientific evaluation procedure was initiated.
3) On 15 October 2019, 21 April 2020, 12 February 2021, 20 May 2021 and 21 June 2021 EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 17 January 2020, 13 January 2021, 14 May 2021, 21 May 2021, and 24 June 2021 additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) On 03 June 2021, EFSA received a letter from the European Commission with the request for a scientific opinion on frozen and dried formulations from whole yellow mealworm (*Tenebrio molitor* larva) as a novel food.
6) During its meeting on 07 July 2021, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of frozen and dried formulations from whole yellow mealworm (*Tenebrio molitor larva*) as a NF pursuant to Regulation (EU) 2015/2283.

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## Abbreviations

| | |
|---|---|
| 3-MCPD | 3-Monochloropropane-1,2-Diol |
| AdDNV | Cricket Densovirus |
| ADF | Acid Detergent Fibre |
| ADL | Acid Detergent Lignin |
| ADME | Absorption, Distribution, Metabolism and Excretion |
| AMCase | Acidic Mammalian Chitinase |
| ANF | Antinutritional Factors |
| AOAC | Association of Official Analytical Chemists |
| aw | water activity |
| BIOHAZ | EFSA Panel on Biological Hazards |
| bw | Body Weight |
| cfu | Colony Forming Units |
| CLA | Conjugated Linoleic Acid |
| CONTAM | EFSA Panel on Contaminants in the Food Chain |
| DIAAS | Digestible Indispensable Amino Acid Score |
| DMSO | Dimethyl Sulfoxide |
| DRVs | Dietary Reference Values |
| FAO | Food and Agriculture Organization of the United Nations |
| FFA | Free Fatty Acids |
| FSANZ | Food Standards Australia New Zealand |
| GC-MS/MS | Gas Chromatography with Tandem Mass Spectrometry |
| GLP | Good Laboratory Practices |
| GMP | Good Manufacturing Practice |
| HACCP | Hazard Analysis Critical Control Points |
| HPAEC-PAD | High Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection |
| HPLC/UV | High Performance Liquid Chromatography/Ultraviolet detector |
| IAC-LC/FLD | Immunoaffinity Chromatography- Liquid Chromatography/Fluorescence Detector |
| ICP-MS | Inductively Coupled Plasma-Mass Spectrometry |
| ICP-OES | Inductively Coupled Plasma Atomic Emission Spectroscopy |
| IIV-19 | Invertebrate iridescent virus 19 |
| IL | Interleukin |
| ISO | International Organization for Standardization |
| ITD | Ion Trap Detector |
| KFDA | Korean Food and Drug Administration |
| LC-MS/MS | Liquid Chromatography/Tandem Mass Spectrometry |
| LOD | Limit of Detection |
| LOQ | Limit of Quantification |
| meq | Milliequivalent |
| MRL | Maximum Residue Level |
| MUFA | Mono-Unsaturated Fatty Acids |
| N | nitrogen |
| ND | not detected |
| NDA | EFSA Panel on Nutrition, Novel Foods and Food Allergens |
| NF | Novel Food |
| nr | not reported |
| NVWA | Dutch Food and Consumer Product Safety Authority |
| OECD | Organization for Economic Co-Operation and Development |
| PA | P-Anisidine Value |

| | |
|---|---|
| PAH | Polycyclic Aromatic Hydrocarbons |
| PBO | Piperonyl Butoxide |
| PCBs | Polychlorinated Biphenyls |
| PCR | Polymerase Chain Reaction |
| PRI | Population Reference Intakes |
| PUFA | Poly-Unsaturated Fatty Acids |
| PV | Peroxide Value |
| SFA | Saturated Fatty Acids |
| Sum of dioxins and dl-PCBs (WHO-TEQ2005) | sum of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls by using toxicity equivalence factors (TEF) set by World Health Organization in 2005. |
| TEF | toxicity equivalent factors |
| TM | Tenebrio Molitor |
| UB | Upper Bound |
| UCT | University of Chemistry and Technology |
| WHO | World Health Organization |
| WHO PCDD, PCDF, PCB TEQ | Sum of Polychlorinated Dibenzo-Para-Dioxins, Polychlorinated Dibenzofurans and Polychlorinated Biphenyls Expressed as World Health Organization Toxic Equivalent |

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

| Amino acids (g/100 g TM dried) | #6 | #7 | #8 | #9 | #10 |
|---|---|---|---|---|---|
| **Alanine**[1] | 3.79 | 3.8 | 3.82 | 3.98 | 3.84 |
| **Arginine**[1] | 2.79 | 2.76 | 2.82 | 2.83 | 2.79 |
| **Aspartic acid**[1] | 4.47 | 4.44 | 4.47 | 4.56 | 4.46 |
| **Cysteine + Cystine**[1] | 0.475 | 0.479 | 0.518 | 0.51 | 0.504 |
| **Glutamic acid**[1] | 5.4 | 5.44 | 5.38 | 5.51 | 5.56 |
| **Glycine**[1] | 2.8 | 2.82 | 2.83 | 2.89 | 2.85 |
| **Histidine***[,1] | 1.62 | 1.64 | 1.62 | 1.66 | 1.62 |
| **Hydroxyproline**[1] | < 0.05 (LOQ) | 0.11 | 0.13 | 0.0706 | 0.147 |
| **Isoleucine***[,1] | 2.21 | 2.25 | 2.25 | 2.26 | 2.25 |
| **Leucine***[,1] | 3.77 | 3.77 | 3.8 | 3.83 | 3.79 |
| **Lysine***[,1] | 3 | 2.98 | 3.04 | 3.04 | 3.02 |
| **Methionine***[,1] | 0.665 | 0.709 | 0.726 | 0.713 | 0.707 |
| **Ornithine**[1] | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 |
| **Phenylalanine***[,1] | 1.88 | 1.88 | 1.86 | 1.88 | 1.85 |
| **Proline**[1] | 3.51 | 3.52 | 3.6 | 3.65 | 3.61 |
| **Serine**[1] | 2.48 | 2.41 | 2.45 | 2.46 | 2.41 |
| **Threonine***[,1] | 2.22 | 2.2 | 2.21 | 2.26 | 2.17 |
| **Tryptophan***[,2] | 0.66 | 0.65 | 0.62 | 0.66 | 0.66 |
| **Tyrosine**[1] | 3.75 | 3.76 | 3.72 | 3.82 | 3.73 |
| **Valine***[,1] | 3.17 | 3.2 | 3.15 | 3.28 | 3.21 |

*: Essential amino acids; LOQ: Limit of Quantification.
1: ISO 13903:2005.
2: Commission Regulation (EC) No 152/2009.

# Appendix B – Comparison of the average amino acid content of TM dried/powder and TM frozen to those of other foods

| Amino acids | Average amino acid content (g/100 g TM dried/ powder) | Average amino acid content (g/100 g TM frozen) ** | Beef and veal (*Bos taurus*) edible flesh[(a)] (g/100g) | Rice (*Oryza* spp.) brown or husked[(a)] (g/100g) | Pork (*Suidae*) edible flesh[(a)] (g/100g) | Chicken (*Gallus gallus*) edible flesh[(a)] (g/100g) | Wheat (*Triticum* spp.) whole grain[(a)] (g/100g) |
|---|---|---|---|---|---|---|---|
| **Alanine** | 3.85 | 1.094 | 1.033 | 0.474 | 0.654 | 0.682 | 0.472 |
| **Arginine** | 2.80 | 0.796 | 1.118 | 0.650 | 0.756 | 1.114 | 0.602 |
| **Aspartic acid** | 4.48 | 1.275 | 1.590 | 0.808 | 1.060 | 1.834 | 0.644 |
| **Cystine** | nr | nr | 0.230 | 0.084 | 0.133 | 0.262 | 0.332 |
| **Cysteine + Cystine** | 0.50 | 0.141 | nr | nr | nr | nr | nr |
| **Glutamic acid** | 5.46 | 1.553 | 2.703 | 1.622 | 1.718 | 3.002 | 3.900 |
| **Glycine** | 2.84 | 0.807 | 0.86 | 0.393 | 0.676 | 1.059 | 0.512 |
| **Histidine*** | 1.63 | 0.464 | 0.603 | 0.197 | 0.391 | 0.525 | 0.299 |
| **Hydroxyproline** | 0.11 | 0.033 | nr | nr | nr | nr | nr |
| **Isoleucine*** | 2.24 | 0.638 | 0.852 | 0.300 | 0.608 | 1.069 | 0.426 |
| **Leucine*** | 3.79 | 1.079 | 1.435 | 0.648 | 0.897 | 1.472 | 0.871 |
| **Lysine*** | 3.02 | 0.858 | 1.573 | 0.299 | 0.961 | 1.590 | 0.374 |
| **Methionine*** | 0.70 | 0.200 | 0.478 | 0.183 | 0.321 | 0.502 | 0.196 |
| **Ornithine** | < 0.05 (LOQ) | < 0.05 (LOQ) | nr | nr | nr | nr | nr |
| **Phenylalanine*** | 1.87 | 0.532 | 0.778 | 0.406 | 0.496 | 0.800 | 0.589 |
| **Proline** | 3.58 | 1.018 | 0.668 | 0.369 | 0.542 | 0.829 | 1.289 |
| **Serine** | 2.44 | 0.695 | 0.713 | 0.427 | 0.496 | 0.781 | 0.600 |
| **Threonine*** | 2.21 | 0.629 | 0.812 | 0.307 | 0.583 | 0.794 | 0.382 |
| **Tryptophan*** | 0.65 | 0.185 | nr | nr | 0.162 | 0.205 | nr |
| **Tyrosine** | 3.76 | 1.069 | 0.637 | 0.275 | 0.426 | 0.669 | 0.391 |
| **Valine*** | 3.20 | 0.911 | 0.886 | 0.433 | 0.616 | 1.018 | 0.577 |

nr: results not reported.
*: Essential amino acids; LOQ: Limit of Quantification.
**: Values calculated from TM dried (average dry matter content: TM dried = 98.14%, TM frozen = 27.92%, from Table 1).
(a): Values from (FAO, 1970).

## Appendix C – Detailed fatty acid profile analysis of TM dried

| Fatty acids (% total fatty acids)[a] | #6 | #7 | #8 | #9 | #10 |
|---|---|---|---|---|---|
| **Total Saturated (SFA)** | 24.6 | 24.5 | 25.1 | 24.9 | 24.8 |
| **C4:0** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C6:0** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C8:0** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C10:0** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C11:0** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C12:0** | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| **C13:0** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C14:0** | 2.5 | 2.5 | 2.4 | 2.3 | 2.5 |
| **C15:0** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| **C16:0** | 18.5 | 18.4 | 19.1 | 19.0 | 18.8 |
| **C17:0** | 0.1 | 0.1 | 0.1 | 0.2 | 0.2 |
| **C18:0** | 2.7 | 2.6 | 2.8 | 2.8 | 2.7 |
| **C19:0** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C20:0** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| **C21:0** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C22:0** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C23:0** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C24:0** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **Total Monounsaturated (MUFA)** | 49.0 | 49.0 | 48.6 | 48.6 | 48.8 |
| **C10:1** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C12:1** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C14:1 (n-9t)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C14:1 (n-9c)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C15:1 (n-10t)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C15:1 (n-10c)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C16:1 (n-9t)** | 0.7 | 0.7 | 0.6 | 0.6 | 0.7 |
| **C16:1 (n-7t)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C16:1 (n-9c)** | 1.8 | 1.8 | 1.7 | 1.7 | 1.7 |
| **C17:1 (n-7t)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C17:1 (n-7c)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C18:1 (n-11t)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C18:1 (n-9t)** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| **C18:1 (n-7t)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C18:1 (n-11c)** | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 |
| **C18:1 (n-9c)** | 45.6 | 45.6 | 45.4 | 45.4 | 45.5 |
| **C18:1 (n-7c)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C18:1 (n-6c)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C19:1 (n-10t)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C19:1 (n-7t)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C20:1 (n-9t)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C20:1 (n-9c)** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| **C22:1 (n-11c)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C22:1 (n-9t)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C22:1 (n-9c)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C24:1** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **Total Polyunsaturated (PUFA)** | 26.4 | 26.5 | 26.3 | 26.5 | 26.3 |
| **C16:2 (n-4)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |

| Fatty acids (% total fatty acids)[a] | #6 | #7 | #8 | #9 | #10 |
|---|---|---|---|---|---|
| **C16:3 (n-4)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C18:2 (5,9)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C18:2 (n-6t)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C18:2 (n-6c)** | 23.8 | 23.8 | 23.8 | 23.9 | 23.7 |
| **9(Z), 11(E) C18:2 (CLA)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **10(E), 12(Z) C18:2 (CLA)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C18:3 (5, 9, 12)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C18:3 (n-6)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C18:3 (n-4)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C18:3 (n-3)** | 2.4 | 2.4 | 2.4 | 2.4 | 2.4 |
| **C18:4 (n-3)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C20:2** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| **C20:3 (5, 11, 14)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C20:3 (n-6t)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C20:3 (n-6c)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C20:3 (n-3c)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C20:4 (n-6c)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C20:4 (n-3c)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C20:5 (n-3)** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| **C22:2 (n-6c)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C22:3** | <0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C22:4 (n-6c)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C22:5 (n-6)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C22:5 (n-3)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **C22:6 (n-3)** | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| **Total*Trans*** | 0.9 | 0.8 | 0.7 | 0.7 | 0.8 |
| **Total Omega 3** | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| **Total Omega 6** | 23.8 | 23.8 | 23.8 | 24.0 | 23.8 |

(a): Analysed by ISO 12966-2/4.
CLA: Conjugated Linoleic Acid.

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

Information provided in this Annex is shown in an Excel file (downloadable at https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2021.6778#support-information-section).