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

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### Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on dried 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 is the thermally dried yellow mealworm, either as whole dried insect or in the form of powder. The main components of the NF are protein, fat 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 that there are no safety concerns regarding the stability of the NF if the NF complies with the proposed specification limits during its entire shelf life. The NF has a high protein content, although the true protein levels in the NF are overestimated when using the nitrogen-to-protein conversion factor of 6.25, due to the presence of non-protein nitrogen from chitin. The applicant proposed to use the NF as whole, dried insect in the form of snacks, and as a food ingredient in a number of food products. The target population proposed by the applicant is the general population. The Panel notes that considering the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous. The 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.

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

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## 1. Introduction

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

On 13 February 2018, the company SAS EAP Group submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283 to place on the market dried yellow mealworm (*Tenebrio molitor* larva) as a novel food (NF).

On 03 July 2018 and in accordance with Article 10(3) of Regulation (EU) 2015/2283, the Commission asked the European Food Safety Authority to provide a scientific opinion on dried yellow mealworm (*Tenebrio molitor* larva).

## 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<sup>1</sup>.

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: analyses of contaminants in the NF, detailed description of the drying process, analytical data on chitin levels, data on the oxidative and microbiological status of the NF during storage, and allergenicity testing using the NF as testing material.

### 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 the whole, thermally dried *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 of yellow mealworms and consists mainly of protein, fat and fibre. The NF is proposed to be consumed as whole, dried insect or in the form of powder, added to various products such as energy bars, pasta and biscuits. Products with the NF can be consumed by the general population.

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

### 3.2. Identity of the NF

The NF is the whole, thermally dried yellow mealworm, either 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 *Tenebrio molitor* larvae 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 whole, thermally dried *T. molitor* larva (blanched, oven-dried larva) and as powder of whole, thermally dried *T. molitor* larva (blanched, oven-dried, ground larva). The entire mealworms are meant for human consumption, no parts are removed. 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. Moreover, the implemented safety management system for the production of the NF follows the requirements of the ISO 22000:2005 standard. The initial livestock of *T. molitor* was obtained from the Office Pour les Insectes et leur Environnement (OPIE), France. The production process can be divided into three main parts, i.e. farming, harvest 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 consequently grow separately. After being hatched from the eggs, the light yellow-brown larvae grow in regularly disinfected containers made of certified food-contact hard-type plastic (high-density polyethylene). Ingestion of soft-type plastic materials by larvae of the Tenebrionidae family has been reported (Brandon et al., 2018; Yang et al., 2018). The breeding containers used for production are, however, made of a hard plastic, reducing the probability of plastic ingestion. The applicant reported that no antimicrobial substances or veterinary medicinal products are used during the rearing of the larvae.

The applicant documented that the feed used is plant-derived and consists of materials such as vegetables and cereal flour. Levels of heavy metals, pesticide residues, and other undesirable compounds (e.g. polychlorinated biphenyls (PCBs), dioxins) are monitored in the feed since yellow mealworms can bioaccumulate such chemical agents (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). Water is provided to the larvae through some components of the feed (e.g. vegetables) and air humidity which is controlled by using appropriate ventilation systems.

*T. molitor* can be infected by parasites, entomopathogenic fungi and viruses (Vigneron et al., 2019). The applicant stated there are measures in place to monitor the presence of the tapeworms (class: cestoda) *Hymenolepis diminuta* ('rat tapeworm'), *Hymenolepis nana* and Newcastle disease virus. All three are zoonotic agents and may cause mild symptoms in humans. *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 at species or family level, and are not pathogenic for humans or other vertebrates (EFSA Scientific Committee, 2015).

Mechanical sieving is used to harvest the larvae (~ 11 weeks old), separating them from the substrate, exuvia and faeces. Decayed larvae, which have a darker colour compared to the alive larvae, are removed by visual inspection. After the harvest, a minimum 24-h fasting step is implemented, to allow the larvae to discard their bowel content.

The post-harvest processing includes rinsing of the larvae with water, killing of the larvae by blanching (immersion for 1–5 min in boiling water), draining, dehydration of the larvae by ventilation (see below), packaging and storage. Killing by boiling 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).

Dehydration of the larvae takes place in a ventilated oven at 78°C (duration may vary depending on ambient conditions and volume of insects to be dried), resulting in a final product with  $a_w < 0.6$ . Two formulations of the NF are produced, i.e. whole, dried larvae and powder of whole, dried and ground larvae. The powder is obtained via mechanical grinding of the whole, dried larvae. The grinding step, which may release from the larval gut any remaining microbiota, can further affect the microbial status of the larval powder (Klunder et al., 2012; Stoops et al., 2016). The NF is stored in hermetically closed packaging at room temperature.

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 formulations, i.e. (a) whole, thermally dried yellow mealworms and (b) powder from whole, thermally dried yellow mealworms. For all parameters, at least three to five batches were analysed. Considering the production process, the Panel considers the two formulations of the NF as 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 it 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 the EFSA Scientific Committee (2011), meaning that all its constituents cannot be fully identified and/or characterised (EFSA NDA Panel, 2016).

The NF mainly consists of protein, fat and dietary fibre (mainly chitin). The results of the proximate analysis of the NF are presented in Table 1. The amino acid, fatty acid, vitamin and mineral compositions are reported in the section '3.9 Nutritional information'.

**Table 1:** Proximate analysis of the NF (whole, dried yellow mealworm)

Parameter (unit)	Batch number					Analytical method
	#1	#2	#3	#4	#5	
<b>Crude protein (g/100 g of NF)</b>	57.2	55.5	61.4	58.9	58.8	Kjeldahl (N × 6.25)
<b>Fat (g/100 g of NF)</b>	28.4	31.6	22.7	27.6	23.2	Gravimetric method
<b>Digestible carbohydrates (g/100 g of NF)</b>	1.8	1.1	< 0.1	3.7	7.7	Calculation by difference <sup>(a)</sup>
<b>Sugars (g/100 g of NF)</b>	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	IC-PAD <sup>(b)</sup> , internal adaptation
<b>Dietary fibre<sup>(c)</sup> (g/100 g of NF)</b>	6.4	6.7	6.4	4.1	4.7	AOAC 985.29, internal adaptation according to AOAC 991.43
<b>Ash (g/100 g of NF)</b>	3.79	3.71	4.31	3.94	4.24	Gravimetric method
<b>Moisture (g/100 g of NF)</b>	2.3	1.4	6.1	1.7	1.4	Gravimetric method
<b>Energy (kcal/100 g of NF)</b>	505	524	462	507	484	Regulation (EU) 1169/2011
<b>Energy (kJ/100 g of NF)</b>	2,107	2,186	1,934	2,120	2,025	Regulation (EU) 1169/2011

(a): Digestible carbohydrates = 100 – (crude protein + fat + dietary fibre + ash + moisture).

(b): IC-PAD: ion chromatography-pulsed amperometric detection.

(c): Chitin is the main form of dietary fibre in the NF; AOAC: Association of Official Agricultural Chemists.

The Panel notes that there is a variation of the values of some proximate parameters, but this can be expected since the NF is produced using whole insects and such variations in composition may occur. The values may depend on the rearing conditions (feed, exact developmental stage at the time of harvesting, ambient conditions) (Rumpold and Schlüter, 2013a; Oonincx et al., 2015), as well as on specific aspects of the processing methods.

Regarding the crude protein content of the NF, the Panel notes that recent literature (Janssen et al., 2017b) suggests that it is possibly overestimated when using the nitrogen-to-protein conversion



factor of 6.25, mainly due to the presence of chitin. This issue will be addressed in detail in the Section '3.9 Nutritional information'.

Chitin is 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  $\beta$ -(1,4)-linked 2-amino-2-deoxy- $\beta$ -D-glucopyranose and 2-acetamido-2-deoxy- $\beta$ -D-glucopyranose residues (Muzzarelli, 1973; Roberts, 1992). The physicochemical nature of chitin is intrinsically related to its source (Kumirska et al., 2011). After EFSA's request, the applicant provided analytical data on the levels of chitin in 5 independently produced batches of the NF (powder) (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 based on an in-house implementation of the protocol described by Hahn et al. (2018), in which chemical treatment (based on determination of the N-acetyl groups) is used to estimate the chitin content of the larval powder. The detailed in-house implemented protocol as well as the results of its validation procedures have been provided by the applicant. The reported average chitin content in the larval powder was found to be  $6.42 \pm 0.28$  g/100 g which is comparable to the values reported for dietary fibre in the NF (Table 1).

**Table 2:** Chitin content of the NF (larval powder)

	Batch number				
	#2	#6	#7	#8	#9
<b>Chitin (g/100 g of NF)</b>	6.21	6.86	6.44	6.16	6.42

Levels of cadmium and lead and, after EFSA's request, of arsenic and mercury in the NF were provided by the applicant (Table 3). The applicant compared the values to the maximum levels for other foods as set in Regulation (EC) No 1881/2006 (2006). 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.

After EFSA's request, further analytical data on the levels of aflatoxins B1, B2, G1, G2, deoxynivalenol, fumonisins, zearalenone and ochratoxin A for both NF formulations have been provided (Table 3).

**Table 3:** Heavy metal and mycotoxin levels in the NF (whole, dried larvae and/or larval powder)

Parameter	Analytical method	Batch number							
		#4	#9	#10	#11	#12	#13	#14	#15
<b>NF Formulation</b>		Whole	Whole	Whole	Whole	Whole	Whole	Whole	Powder
<b>Heavy metals (mg/kg)</b>									
<b>Arsenic (As)</b>	Internal adaptation of EN 15763:2010, ICP-MS <sup>(a)</sup>	/	0.24	0.29	0.18	0.21	/	/	/
<b>Mercury (Hg)</b>		/	0.019	0.044	0.012	0.01	/	/	/
<b>Lead (Pb)</b>		< 0.02	/	/	/	/	< 0.02	< 0.02	< 0.075
<b>Cadmium (Cd)</b>		0.069	/	/	/	/	0.035	0.051	/
<b>Mycotoxins (<math>\mu</math>g/kg)</b>									
<b>Aflatoxin B1</b>	Internal adaptation of EN 14123	< 1	/	< 0.1	/	/	/	< 0.1	< 0.1
<b>Aflatoxin B2</b>		< 1	/	< 0.1	/	/	/	< 0.1	< 0.1
<b>Aflatoxin G1</b>		< 1	/	< 0.1	/	/	/	< 0.1	< 0.1
<b>Aflatoxin G2</b>		< 1	/	< 0.1	/	/	/	< 0.1	< 0.1
<b>Aflatoxins (Sum of B1, B2, G1, G2)</b>		< 4	/	/	/	/	< 0.4	< 0.4	/
<b>Ochratoxin A</b>		< 1	/	0.2	/	/	/	< 0.2	< 0.2
<b>Deoxynivalenol</b>	Internal method, LC-MS/MS <sup>(b)</sup>	< 20	/	< 20	/	/	/	< 20	< 20
<b>Fumonisin B1</b>		/	< 20	< 20	< 20	< 20	/	/	/
<b>Fumonisin B2</b>		/	< 20	< 20	< 20	< 20	/	/	/
<b>Zearalenone</b>		/	< 10	< 10	< 10	< 10	/	/	/

/ = data not provided.

(a): ICP-MS: inductively coupled plasma mass spectrometry.

(b): LC-MS/MS: liquid chromatography–tandem mass spectrometry.

The applicant did not provide analytical data on the levels of dioxins and dl-PCBs in the NF but stated that the levels of these compounds are regularly controlled in the feed and provided analytical certificates in which the respective compounds were below the limit of detection (LOD) of the analytical methods used.

Analytical data of the pesticide levels (organochlorine pesticides & pyrethroids, organophosphate pesticides) for five independently produced batches of the NF have been provided. The results showed that the tested pesticide levels in the NF are below the limits of quantification (LOQ) of the implemented method (ASU L00.00-34).

Given the vegetable origin of the substrate and the absence of prion or prion-related protein 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 microbiological data on nine independently produced batches of the NF (for both formulations). The Panel notes that the applicant did not provide the actual values of the microbiological parameters. Following EFSA's request, the applicant explained that the actual values could not be calculated since the samples had been diluted too much, and instead, the quantification limits as defined by the dilutions used upon the analyses were provided.

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

Parameter	Units	Batch number								
		#15	#16	#17	#18	#19	#20	#4	#21	#22
<b>NF Formulation</b>		Powder						Whole, dried larvae		
<b>Total aerobic colony count</b>	CFU/g	< 10,000	< 1,000	< 4,000	12,000	< 10,000	< 10,000	/	< 100,000	< 1,000
<b>Yeasts</b>	CFU/g	< 1,000	/	/	/	< 1,000	/	/	< 100	/
<b>Moulds</b>	CFU/g	< 1,000	< 10	< 10	40	< 1,000	40	< 10	/	< 10
<b><i>Salmonella</i> spp.</b>	In 25 g	n.d.	n.d.	n.d.	/	n.d.	n.d.	n.d.	n.d.	n.d.
<b><i>Listeria monocytogenes</i></b>	In 25 g	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<b>Sulfite-reducing Anaerobes</b>	CFU/g	/	< 10	< 10	< 10	/	< 10	< 10	< 10	< 10
<b><i>Bacillus cereus</i></b>	CFU/g	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100
<b>Presumptive Enterobacteriaceae</b>	CFU/g	< 10	< 10	< 10	< 10	/	/	< 10	< 10	< 10
<b>Coagulase positive Staphylococci</b>	CFU/g	/	< 100	< 100	/	/	/	< 100	/	< 100
<b><i>Staphylococcus aureus</i></b>	CFU/g	< 10	/	/	/	< 10	/	/	/	/
<b><i>Clostridium perfringens</i></b>	CFU/g	< 10	/	/	/	< 10	/	/	/	/
<b><i>Escherichia coli</i></b>	CFU/g	< 10	/	/	/	< 10	/	/	< 10	/
<b><i>Cronobacter</i> spp. (<i>Enterobacter sakazakii</i>)</b>	In 10 g	/	/	/	/	/	/	n.d.	/	n.d.

/: data not provided; n.d.: not detected; CFU: colony forming units.

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 10 batches of the NF (powder) which have been either analysed immediately after manufacturing (0 months) or analysed after having been stored at room temperature for 24 months (Table 5). The Panel notes that the five NF batches analysed at time 24 are not the same five NF batches analysed at time 0. Furthermore, since the applicant did not provide the actual values of the microbiological parameters, but instead the



quantification limits as defined by the dilutions used, the Panel can comment only that the microbiological values of most of the analysed samples do not exceed the given specification limits.

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

Parameter (unit)	Batch number									
	#15	#16	#17	#18	#23	#22	#24	#25	#26	#27
<b>Time (months)</b>	0					24				
<b>Aerobic plate count (30°C) (CFU/g)</b>	< 10,000	< 1,000	< 4,000	12,000	< 10,000	< 1,000	< 1,000	< 1,000	< 1,000	< 1,000
<b>Yeasts (CFU/g)</b>	< 1,000	/	/	/	< 1,000	/	/	/	/	/
<b>Moulds (CFU/g)</b>	< 1,000	< 10	< 10	40	< 1,000	< 10	< 10	< 10	< 40	< 10
<b>Sulfite-reducing Anaerobes (CFU/g)</b>	/	< 10	< 10	< 10	/	< 10	< 10	< 10	< 10	< 10
<b>Bacillus cereus (CFU/g)</b>	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100
<b>Listeria monocytogenes in 25 g</b>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<b>Presumptive Enterobacteriaceae (37°C) (CFU/g)</b>	< 10	< 10	< 10	< 10	< 40	< 10	< 10	< 10	< 10	< 10
<b>Salmonella in 25 g</b>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<b>Coagulase positive Staphylococci</b>	< 10	< 100	< 100	< 100	< 10	< 100	< 100	< 100	< 100	< 100

/: data not provided; n.d.: not detected; CFU: colony forming units.

After EFSA's request, the applicant provided analytical data on the oxidative status of fats in the NF (larval powder). Peroxide value (PV), *p*-anisidine value (AV), total acidity have been determined and Totox values have been calculated (Table 6). The data provided cover a period of at least 24 months which is the proposed shelf life. The Panel notes that no monitoring results of specific NF batches over time were provided.

**Table 6:** Oxidative status of fat in the NF (powder) during the proposed shelf life

Parameter (unit)	Batch number														
	#28	#16	#29	#30	#17	#31	#32	#33	#34	#35	#36	#37	#38	#39	
<b>Time (months)</b>	0							4	9	14	19	24			
<b>p-anisidine value</b>	0.8	2.3	1.1	< 0.5	< 0.5	1.1	1.2	0.9	0.9	0.8	0.5	0.6	1.0	0.5	
<b>Peroxide value (meq O<sub>2</sub>/kg fat)<sup>(a)</sup></b>	6.1	1	1.3	1.8	1.9	13.2	11.1	12.2	7.5	5.9	16.3	13.8	14.2	16.4	
<b>Free fatty acids- Total acidity as oleic acid (g/100 g fat)</b>	1.54	0.52	0.6	0.67	0.56	1.55	1.7	2.66	4.82	4.74	1.1	1.66	1.79	1.1	
<b>Totox value<sup>(b)</sup></b>	13	4.3	3.7	3.6	3.8	27.5	23.4	25.3	15.9	12.6	33.1	28.2	29.4	33.3	

(a): Meq: milliequivalents.

(b): Totox value = AV + 2PV.

Regarding the relatively high peroxide value (PV) of 6.1 meq O<sub>2</sub>/kg fat in 'lot 1-190718' at t = 0 when compared to the rest of the analysed samples, the applicant indicated that such variation could be due to temperature fluctuations during the rearing period. The Panel is of the view that such variation is more probably related to the drying step of the production process (changes of duration/ volume of insects dried) and that the apparent increase of the PVs during time indicates oxidation of the fat in the NF during storage. Additionally, the observed fluctuation of the PV values, and consequently of the Totox values can also be due to variations in the drying step of the production process. However, no further conclusions can be made since the data in Table 5 for time = 0 months and time = 24 months correspond to different batches of the NF. The applicant proposed

the specification limit of PV of  $\leq 5$  meq O<sub>2</sub>/kg fat. The Panel notes that most of the PV values of the analysed NF batches during the proposed shelf life of 24 months (Table 6), do not comply with the proposed limit. After EFSA's request, the applicant clarified that stricter control of the drying step will be implemented so that the NF complies with the proposed specification limit. The Panel considers that a maximum level of PV  $\leq 5$  meq O<sub>2</sub>/kg fat does not raise safety concerns. The shelf-life of 24 months proposed by the applicant may be too long, considering the resulting values for the time point t = 24 months. The Panel cannot conclude on the NF's shelf life since appropriate data for intermediate timespans have not been made available by the applicant.

Since the NF is going to be used as an ingredient for the manufacturing of other foods, the applicant was asked by EFSA to investigate its stability when used as an ingredient in the intended-for-use matrices (see Section 3.7.2 Proposed uses and use levels). In particular, the applicant tried to address the formation of acrylamide and other processing contaminants, the evolution of microbial contaminants and the oxidative stability of fats.

The applicant provided acrylamide levels for two bakery products (crackers with 10% inclusion of the NF (larval powder). The cracker batch baked at 180°C for 22 mins contained 577 µg/kg acrylamide and the cracker batch baked at 160°C for 30 min contained 77 µg/kg acrylamide. However, appropriate control samples (crackers produced in the same way but without the NF) were not provided and no conclusion on the impact of the NF on acrylamide formation could be drawn. Furthermore, for one cracker with the NF as ingredient, the applicant reported levels of 3-MCPD and its esters, glycidyl esters and glycidol, aromatic amines and furans but since results of control samples were not provided, no conclusion could be made regarding the contribution of the NF to the concentrations of these contaminants.

The applicant provided also microbiological results for five cereal bar batches with the NF as ingredient (10%, powder), all produced with different recipes. Also results on the oxidative status for 5 cracker batches with the NF (10% inclusion, powder), produced with different recipes have been provided. The Panel notes the limited number of analysed samples, the absence of control samples and concludes that no conclusions can be made regarding the stability of the NF when used as ingredient in other foodstuffs.

Despite the limitations of the data provided on the stability of the NF, the Panel notes that the microbiological values of most of the analysed batches do not exceed the given specifications. The Panel notes that most of the PV values of the analysed NF batches during the proposed shelf life of 24 months (Table 6), do not comply with the proposed specification limit. Additionally, the Panel notes that the analytical data regarding the putative formation of contaminants due to the use of NF as an ingredient in the intended-for-use matrices are limited, and no conclusion can be drawn due to the absence of proper control samples. The Panel notes that the food items containing the NF have to comply with existing legislative limits, such as the benchmark levels of acrylamide in bakery products established by Regulation (EU) 2017/2158 (2017). The Panel could not fully conclude on the stability of the NF based on the submitted data. However, provided that the specifications are met also at the end of shelf life, and that products containing the NF are compliant with respective legislative limits on process formed contaminants, the stability data do not raise safety concerns.

### 3.5. Specifications

The specifications of the NF are indicated in Table 7.

**Table 7:** Specifications of the NF

<b>Description:</b>			
a) whole, thermally dried yellow mealworms ( <i>Tenebrio molitor</i> larvae)			
b) powder of whole, thermally dried yellow mealworms ( <i>Tenebrio molitor</i> larvae)			
<b>Parameters</b>	<b>Unit</b>	<b>Specification</b>	<b>Method of analysis</b>
<b>Moisture</b>	% w/w	1–8	Thermogravimetry
<b>Crude protein (N × 6.25)</b>	% w/w	56–61	Kjeldahl (N × 6.25)
<b>Digestible Carbohydrates</b>	% w/w	1–6	Calculation <sup>(a)</sup>
<b>Fat</b>	% w/w	25–30	Gravimetric method

of which saturated	% w/w	4–9	GC-FID <sup>(b)</sup>
<b>Peroxide value</b>	Meq O <sub>2</sub> /kg fat	≤ 5	Titrimetry
<b>Dietary fibre</b>	% w/w	≤ 7	AOAC 985.29, internal adaptation according to AOAC 991.43
<b>Chitin</b>	% w/w	≤ 7	Hahn et al. (2018), internal adaptation
<b>Heavy metals</b>			
Lead	mg/kg	≤ 0.075	ICP-MS <sup>(c)</sup> , internal adaptation of EN 15763:2010
Cadmium	mg/kg	≤ 0.1	ICP-MS, internal adaptation of EN 15763:2010
<b>Mycotoxins</b>			
Aflatoxins (Sum of B1, B2, G1, G2)	µg/kg	≤ 0.4	internal adaptation of EN 14123:2009 (HPLC-fluorescence) <sup>(d)</sup>
Deoxynivalenol	µg/kg	≤ 20	LC-MS/MS <sup>(e)</sup>
Ochratoxin A	µg/kg	≤ 1	Internal adaptation of EN 14132:2009 (HPLC-fluorescence)
<b>Microbiological</b>			
Total aerobic colony count	CFU/g	≤ 10 <sup>5</sup>	Plate Counting Method NF EN ISO 4833-1:2013
Yeasts and Moulds	CFU/g	≤ 100	Plate Counting Method NF V 08-036:2003
<i>Escherichia coli</i>	CFU/g	≤ 50	Plate Counting Method ISO 16649-2:2001
<i>Salmonella</i> spp.		Not detected in 25 g	Qualitative Method BIO-RAD Rapid Salmonella
<i>Listeria monocytogenes</i>		Not detected in 25 g	Qualitative Method AFNOR: AES 10/03-09/00
Sulfite-reducing Anaerobes	CFU/g	≤ 30	NF ISO 15213:2003
<i>Bacillus cereus</i> (presumptive)	CFU/g	≤ 100	Plate Counting Method Internal adaptation of NF EN ISO 7932:2004
Enterobacteriaceae (presumptive)	CFU/g	< 10	Plate Counting Method NF V 08-054:2009
Coagulase-positive staphylococci	CFU/g	≤ 100	Plate Counting Method NF EN ISO 6888-1:2018

(a): Digestible carbohydrates = 100 – (crude protein + fat + dietary fibre + ash + moisture).

(b): GC-FID: gas chromatography with flame-ionisation detection.

(c): ICP-MS: inductively coupled plasma mass spectrometry.

(d): HPLC: high-performance liquid chromatography.

(e): LC-MS/MS: liquid chromatography–tandem mass spectrometry; CFU: colony forming units.

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 the 1st of May 2017, *T. molitor* larvae is among the insect species that can be legally introduced in the Swiss market as food (whole, chopped or ground).

### 3.7. Proposed uses and use levels and anticipated intake

#### 3.7.1. Target population

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

#### 3.7.2. Proposed uses and use levels

The NF (whole, dried larvae or larval powder) is proposed to be used as an ingredient in several food products. These food products defined using the FoodEx2 hierarchy, and the maximum use levels are reported in Table 8.

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

FoodEx2 level	FoodEx2 code	Food category	Max use level (g NF/100 g)
L3	A06HL	Snacks other than chips and similar	100
L4	A03SA	Protein and protein components for sports people	10
L3	A009V	Biscuits	10
L4	A03VM	Legumes-based dishes	10
L4	A007S	Pasta-based dishes, uncooked	10

#### 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). The lowest and highest mean and 95th percentile anticipated daily intakes 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 estimates 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 <sup>(b)</sup>	Highest <sup>(b)</sup>	Lowest <sup>(c)</sup>	Highest <sup>(c)</sup>
Infants	< 1	0	76.5	0	419.1
Young children <sup>(a)</sup>	1 to < 3	10.4	216.2	60.0	901.5
Other children	3 to < 10	1.4	248.3	7.5	768.6
Adolescents	10 to < 18	0.3	103.5	2.1	451.4
Adults <sup>(d)</sup>	≥ 18	1.5	40.8	10.4	203.4

bw: body weight.

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

(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 8/1/2020. The lowest and the highest averages observed among all EU surveys are reported in these columns.

(c): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 8/1/2020. The lowest and the highest P95th observed among all EU surveys are reported in these columns (P95th based on less than 60 individuals are not considered).

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

#### 3.7.4. Estimate of exposure to undesirable substances

Based on the highest P95th intake estimate (Table 9), EFSA calculated the estimate of exposure to undesirable substances (heavy metals, toxins), for all population groups. The specification limits (Table 7) were used as maximum values for the concentration of the undesirable substances. When

specification limits for a substance of possible concern have not been proposed, the maximum values reported for the analysed batches were used. Consumption of the NF under the proposed uses and use levels does not contribute significantly to the overall exposure to the analysed undesirable substances through diet.

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

The applicant provided no ADME data for the NF.

### 3.9. Nutritional information

The applicant provided a nutritional analysis of the NF which consists mainly of protein, fat, dietary fibre (mainly chitin) and inorganic matter. The energy value of the NF is on average 2,074 kJ (496 kcal)/100 g (Table 1). Analytical data on the amino acid composition, the fatty acid content, minerals and vitamins in the NF have been provided for a number of different batches of the NF formulations. For all parameters, three to five batches were analysed.

The NF contains on average 58.4 ( $\pm$  2.2) g crude protein per 100 g, calculated using the conventional nitrogen-to-protein conversion factor of 6.25. The Panel notes that the use of the conventional factor overestimates the level of true protein content in 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 44.5 g/100 g (23.8% lower than with a conversion factor of 6.25). For regulatory purposes for nutrition 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 (2011) on the provision of food information to consumers]. More accurate conversion factors have been reported for insects (see above) and other foodstuffs (FAO, 2013).

The applicant quantified the amino acids in five batches of the NF according to ISO 13903:2005 and/or Commission Regulation (EC) No 152/2009 (2009) (Appendix A) and compared the amino acid profile of the NF to the amino acid profile of other foods (FAO, 1970) (Appendix B). The content of all individual amino acids is higher than those of the foods used for comparison (barley, fish, brewer's yeast, beef/veal, crustaceans) except for lysine, which is slightly higher in brewer's yeast.

To investigate further the nutritional quality of the protein, the applicant did not conduct any protein digestibility studies. The applicant referred instead to the study of Marono et al. (2015), who reported an *in vitro* crude protein digestibility of 66.12% ( $\pm$  0.38) measured after enzymatic digestion with pepsin and trypsin-enriched pancreatin, of six dried *T. molitor* larvae meals obtained from different producers. The authors also reported a negative correlation ( $p < 0.05$ ) between the crude protein digestibility and the chitin content.

In the study of Jensen et al. (2019), the protein digestibility-corrected amino acid score (PDCAAS) of freeze-dried yellow mealworm, considering the amino acid reference profile for children aged 0.5–3 years (FAO, 2013) and true crude protein faecal digestibility in rats, was found to be 76%. This value is comparable to the PDCAAS value of 73% reported on average for vegetables (Suárez et al., 2006) but lower than the one of 92% for beef and 91% for soy (Schaafsma, 2000), although care has to be taken in comparing values from different studies. The limiting amino acids in freeze-dried yellow mealworm were the sulfur-containing ones.

In another study, an apparent ileal digestibility (AID) of amino acids of 89–90% was found in growing pigs fed dried mealworm (Yoo et al., 2019). The AID of some amino acids was higher in the group fed *Tenebrio molitor* compared with those fed fish meal (i.e. lysine, histidine, arginine, cysteine) or meat meal (i.e. histidine and arginine).

The Panel notes that results reported in the literature for the protein digestibility of dried *T. molitor* larvae differ among the studies. The discrepancies may be caused by the differences in the processing of the yellow mealworm as well as by the use of different techniques (*in vitro* digestibility, *in vivo* faecal digestibility or apparent ileal digestibility of amino acids) and different models (*in vitro* enzymatic digestion, rats, pigs) for the assessment of the protein digestibility (Boye et al., 2012).

Monounsaturated fatty acids comprise on average ~ 47% of the total fatty acids in the NF (~ 12 g/100 g of the NF), followed by ~ 29% polyunsaturated fatty acids (~ 7 g/100 g of the NF), and ~ 24% saturated fatty acids (~ 6 g/100 g of the NF). The average trans fatty acid content is 0.06 g/100g of NF. The principal fatty acid in the NF (determined by GC-FID) is oleic acid C18:1 (n-9c), followed by linoleic acid C18:2 (n-6c) and palmitic acid C16:0. The detailed analytical data on the fatty acid composition can be found in Appendices C and D.

The applicant provided analytical data on the levels of some minerals and vitamins (Table 10). The levels of vitamin D2 and vitamin D3 have been determined only in one batch of the NF (< 0.25 and 0.989 µg/100 g of the NF, respectively), according to EN 12821: 2009-08.

**Table 10:** Levels of micronutrients in the NF

Parameter	Analytical method	Batch Number				
		#9	#10	#12	#40	#41
<b>Minerals (mg/100 g)</b>						
Copper	ICP-MS	1.72	1.69	1.68	1.63	1.6
Iron		5.08	4.45	4.84	4.55	4.26
Magnesium		184	182	196	195	201
Manganese		0.734	0.722	0.68	0.704	0.673
Potassium		1,110	919	872	867	866
Sodium		191	182	193	179	197
Zinc		13	12.7	12.6	12.2	12.8
<b>Vitamins</b>						
Vitamin B12 (µg/100 g)	AOAC 952.20	0.0452	0.0636	0.0495	0.0369	0.0555
Pantothenic acid (mg/100 g)	AOAC 2012.16	5.62	5.36	5.88	5.33	6.05
Riboflavin (mg/100 g)	EN 14152:2006	1.09	0.97	1.06	1.06	1.28

ICP-MS: inductively coupled plasma mass spectrometry.

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

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 Panel considers that chitin is an insoluble fibre that is not expected to be digested in the small intestine of humans to any significant degree. It is also rather resistant to microbial fermentation and therefore assumed to be excreted mainly unchanged. Additionally, the Panel notes that chitin can bind bivalent minerals (Franco et al., 2004; Anastopoulos et al., 2017) possibly affecting their bioavailability, as reported for dietary fibres in general (Baye et al., 2017).

Insects may contain antinutritional factors (ANFs) such as tannins, oxalates, phytate, and hydrogen cyanide (Jonathan et al., 2012; Shantibala et al., 2014), thiaminases (Nishimune et al., 2000), and protein inhibitors (Eguchi, 1993). The applicant determined the concentrations of phytate, oxalates, hydrogen cyanide and phenolics in five independently produced batches of the NF (whole, dried larvae). 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). A detailed description of the methods implemented has been provided by the applicant.

**Table 11:** Levels of antinutrients in the NF

Parameter (unit) analytical method		Batch number					
		#42	#41	#9	#10	#43	#17
<b>Oxalic acid (g/100 g)</b>	Ion chromatography conductivity detector	0.04	0.04	0.04	0.04	/	0.02
<b>Phytic acid (g/100 g)</b>	Ellis et al. (1977)	< 0.14	< 0.14	< 0.14	< 0.14	/	< 0.14
<b>Hydrogen cyanide (mg/kg)</b>	HS-GC/NPD	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5	/
<b>Total polyphenols (mg/kg gallic acid)</b>	Spectrophotometry	7,990	6,890	6,610	6,320	8,170	/

/: data not provided; HS-GC/NPD: headspace gas chromatography with nitrogen-phosphorus 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

Some insect species secrete chemical substances with potentially toxic effects, as part of their defense mechanisms (Dzerefos et al., 2013; Rumpold and Schlüter, 2013b). Regarding *T. molitor*, focus has been given on benzoquinones, substances secreted into the abdominal cavity in adult beetles (Ladisch et al., 1967; Attygalle et al., 1991; Brown et al., 1992). It has been demonstrated that benzoquinones have toxic effects (Wirtz and Fruin, 1982; Lis et al., 2011). The findings refer to *T. molitor* adult insects (beetles) and not to *T. molitor* larvae. Regarding the defensive mechanisms of *T. molitor* larvae, Chiou et al. (1998) reported that acidic methanolic extracts of *T. molitor* larvae have a lethal effect on *T. molitor* adults and on insects of other species. Strongest activity of these compounds has been found to be in late instar larvae. Kotanen et al. (2003) identified the saturated  $\beta$ -carboline, 1,2,3,4, -tetrahydro- $\beta$ -carboline-3-carboxylic acid (THCA), and the essential amino acid, tryptophan, its precursor, as the compounds involved in the defensive mechanism of *T. molitor* larvae. The presence of  $\beta$ -carbolines has been reported in various foodstuffs including, but not limited to, breakfast cereals, fruits, juices and vinegars. Considering the information above, the Panel notes that *T. molitor* larvae should be reared separately from the adults.

Regarding the safety of chitin present in NF, the applicant referred to the EFSA's scientific opinion on the safety of 'chitin-glucan' as a NF ingredient (EFSA NDA Panel, 2010). However, the Panel is of the view that the polymer chitin-glucan cannot be considered as representative of the chitin derived from the *T. molitor* larvae.

Potential adverse health effects of chitin may be related to immunological effects. 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 to allergens. 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. No firm conclusions could be drawn by the Panel since only the abstract was accessible.

The Panel notes that no toxicological studies with the NFs were provided. Instead, the applicant referred to studies available in the literature which investigate the *in vitro* and *in vivo* genotoxicity, subacute toxicity (Han et al., 2014) and subchronic toxicity (Han et al., 2016) of freeze-dried powdered *T. molitor* larvae. The material used in these studies consists of *T. molitor* larvae but was processed differently from the NF. Mealworms in both studies of Han et al. (2014, 2016) were first lyophilised and then sterilised at 115°C for 10 min. The NF was first blanched for 1–5 min at 100°C, and then dried at 78°C for 16 h. Lyophilisation is a method that can preserve up to a high degree the chemical composition of a foodstuff, but slight changes may still be present. However, freeze-drying has low impact on the chemical contaminants' levels (EFSA Scientific Committee, 2015). Furthermore, the rearing conditions of the larvae used in the aforementioned studies are not known. Thus, the view of the Panel is that the material studied by Han et al. (2014, 2016) 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.

Apart from the references provided by the applicant, EFSA identified in the review from Gao et al. (2018) further toxicological studies with *T. molitor* larvae as testing material (Zhou et al., 1996; Chen and Wang, 1997; Yang et al., 1999). However, these studies were not considered further due to poor reporting regarding the testing material and the experimental conditions.

#### 3.10.1. Genotoxicity

The potential *in vitro* and *in vivo* genotoxicity of freeze-dried powdered *T. molitor* larvae (fdTML) was evaluated performing a bacterial reverse mutation test, an *in vitro* chromosome aberration test, and an *in vivo* micronucleus test, that were conducted in compliance with GLP practices, OECD guidelines and KFDA (Korea Food and Drug Administration) guidelines (Han et al., 2014).

Tests on gene mutations (plate incorporation) using *Salmonella* Typhimurium strains TA98, TA100, TA1535, TA1537, and tryptophan-requiring *Escherichia coli* WP2uvrA strain were conducted. In a range-finding experiment, fdTML was not toxic up to the highest tested dose of 5.0 mg/plate.

According to the authors, the results indicated that the fdTML did not increase the number of revertant colonies when compared with the control and therefore it is not mutagenic.

In the *in vitro* chromosome aberration test, Chinese hamster lung cells were incubated with fdTML in the presence or absence of S9 mixture for 6 h and in the absence of S9 mixture for 22 h. Precipitation or turbidity/precipitation of the fdTML were observed at the beginning and at the end of the treatment, at all concentrations. There was also cytotoxicity at the concentration ranges at which the severe turbidity and precipitation of the test articles were shown. Based on these results, the concentration range for the confirmatory test was designed to consider the precipitation of fdTML. The treatment at each concentration was conducted in duplicate and at least 200 well-spread intact metaphases were scored for structural and numerical aberration. Chromatid and chromosome gap were recorded but not included in the calculation of the aberration rates. This study did not show chromosome aberrations of fdTML, at levels up to 5,000 µg/mL, with or without S9 mixture.

An *in vivo* micronucleus test in both male and female mice (six animals/sex/group) treated with fdTML by oral gavage was also performed. In the dose-range finding study performed at up to 2,000 mg/kg body weight (bw), no treatment-related mortality or clinical signs were reported in animals at any doses tested. Therefore, the *in vivo* micronucleus test was conducted at dose levels of 500, 1,000 and 2,000 mg/kg. The fdTML was administered twice at 24-h intervals while positive control (cyclophosphamide monohydrate) was administered once intraperitoneally at 70 mg/kg and animals were sacrificed at approximately 24 h after the last administration. To determine the frequencies of micronucleated polychromatic erythrocytes (PCEs), 2,000 PCEs were scored per animal. There were no statistically significant differences in the number of PCEs in any dose group as compared to the negative control group. This study did not show mutagenic effects of fdTML at levels up to 2,000 mg/kg bw. However, there are insufficient data to confirm the target tissue exposure (absence of bone marrow cytotoxicity and/or lack of evidence of systemic exposure), to allow on the validity of the negative outcome of this study.

Considering the test results provided for the powdered *T. molitor* larvae and the nature, source and production process, the Panel considers that there are no concerns regarding genotoxicity.

### 3.10.2. Subacute toxicity

In the study by Han et al. (2014), fdTML was administered once daily by oral gavage to Sprague–Dawley rats at dose levels of 0, 300, 1,000 and 3,000 mg/kg bw per day, for 28 days. As reported by the authors the study was conducted in compliance with GLP practices, OECD guidelines and KFDA (Korea Food and Drug Administration) guidelines. Mortality, clinical signs, body and organ weights, food consumption, ophthalmology, urinalysis, haematology, serum chemistry, gross- and histopathology were investigated, and no treatment-related changes or findings were observed.

### 3.10.3. Subchronic toxicity

The subchronic (90-day) oral toxicity study by Han et al. (2016) was conducted according to OECD test guideline 408 (OECD, 1998) and in compliance with GLP principles. Groups of 50 male and 50 female Sprague–Dawley rats were administered by gavage fdTML diluted in distilled water, for up to 90 days at dose levels of 0 (vehicle control: distilled water), 300, 1,000 and 3,000 mg/kg per day. Additional rats in control and high-dose group served as study recovery groups. No treatment-related findings have been observed in any of the parameters measured (clinical signs, body and organ weights, food consumption, ophthalmology, urinalysis, haematology and serum chemistry, gross- and histopathology).

### 3.10.4. Summary of toxicological information

No toxicological studies with the NFs were provided. No adverse effects were observed in the toxicological studies available in the literature on freeze-dried yellow mealworms.

### 3.10.5. Human data

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

### 3.11. Allergenicity

The Tenebrionidae mealworm family belongs to the Hexapoda (Insecta) class, one of the four subphyla of Arthropoda. Within arthropods, several allergens have been reported, including tropomyosin (Reese et al., 1999), arginine kinase (Binder et al., 2001) and glutathione S-transferase (Galindo et al., 2001). Furthermore, chitinases, the enzymes that degrade chitin, have been identified as allergens in some insect species (Zhao et al., 2015). The currently available literature on food allergy related to insects is very scarce and the few prevalence studies available are mainly for Asian populations (China and Laos) (Ji et al., 2009; Barennes et al., 2015).

Primary sensitisation to yellow mealworm, the source of the NF, has been recently investigated in humans and animals. In humans, Broekman et al. (2017a,b) studied the risk of allergy to yellow mealworm in four individuals with no allergy to shrimp. Sensitisation to mealworm was investigated using ImmunoCAP blood tests, skin prick tests and basophil activation tests. Two out of the four individuals tested had positive double-blind, placebo-controlled food challenge results to mealworm where clinical manifestations were observed. The authors concluded that exposure to yellow mealworm can induce primary sensitisation and may lead to food and inhalant allergy. Nebbia et al. (2019) studied two allergic individuals with primary sensitisation to yellow mealworm that developed respiratory and oral allergy syndrome. In animals, oral sensitisation to yellow mealworm was described in a mouse model of food allergy where the 'production of IgG1 and IgE antibodies against mealworm proteins was induced in five out of six animals' (Broekman et al., 2017a). In a rat model for toxicity, the dosing of powdered yellow mealworm induced no increase in the levels of serum histamine or IgE (Han et al., 2016).

In addition to primary sensitisation, cross-reactivity to yellow mealworm proteins has also been reported (Verhoeckx et al., 2014). The main reason for cross-reactivity is the high protein homology between phylogenetically related organisms, being evident not only between species within the same subphylum, but also between species from different arthropod subphyla. It includes crustacean species (e.g. shrimp, crab), chelicerates (e.g. mites) and several insect species (Santos et al., 1999; Binder et al., 2001; Galindo et al., 2001; Liu et al., 2009; Lopata et al., 2010; Van Broekhoven et al., 2016; Verhoeckx et al., 2014; De Gier and Verhoeckx, 2018). Yellow mealworm protein can cause adverse reactions in shrimp allergic patients (Broekman et al., 2015, 2017a,b). The applicant provided the study of Velasquez (2015) who investigated the allergenic potential of yellow mealworm larvae using extracts of the NF, and concluded that subjects allergic to arthropods and more specifically to crustaceans, should not consume the NF due to the risk of cross-reactivity. Although clinical studies that evaluate cross-reactivity of mealworm protein in house dust mite allergic individuals are as yet not available, it may occur, as clinically relevant cross-reactivity between shrimp and house dust mite allergens (presumably tropomyosin) has been described (Wittman et al., 1994; Van Ree et al., 1996).

Additional aspects should be taken into consideration depending on the feed substrate used to rear the yellow mealworm, as it might include common allergenic foods (Mancini et al., 2020). The applicant reported that a substrate with gluten-containing grains is used. Gluten was detected in the NF, in a quantity of 5.5 mg/kg. The limit values of 20 and 100 mg/kg of gluten in 'gluten-free' and 'very low gluten' foods has been previously set by regulation. The Panel notes that changes in the feed can possibly introduce additional allergens, including allergens which require mandatory labelling according to Annex II of Regulation (EU) No 1169/2011 (2011), of the NF since traces of the allergens may remain in the gut of yellow mealworms despite the fasting step implemented (Mancini et al., 2020).

A frequently reported cause of allergic symptoms to insects, including the yellow mealworm larvae, relates to occupational exposure (skin contact and inhalation) (Bernstein et al., 1983; Schroeckenstein et al., 1990; Bernstein and Bernstein, 2002).

In addition, in general, food processing may have an influence on allergenicity, and this applies to insect allergens as well (Pali-Schöll et al., 2019), although it cannot always be predicted what the effect of food processing on allergenicity may be (EFSA NDA Panel, 2014). De Gier and Verhoeckx (2018) reported that thermal processing and digestion did not eliminate insect protein allergenicity. Broekman et al. (2015) investigated the effect of thermal processing on mealworm allergenicity using 15 shrimp allergic individuals. The process applied did not lower the allergic potential of mealworm, but it changed its solubility.

The Panel considers that the consumption of the NF may trigger sensitisation to yellow mealworm proteins as well as to tropomyosin from other sources such as crustaceans and mites. The Panel also considers that allergic reactions may occur in subjects allergic to crustaceans. Furthermore, the

Panel notes that additional allergens, may end up in the NF, if these allergens are present in the substrate fed to the insects. This may include allergens listed in the Annex II of Regulation (EU) No 1169/2011 (2011).

#### 4. Discussion

The NF which is the subject of the application is thermally dried yellow mealworms (*Tenebrio molitor* larvae), either 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 NF consists mainly of protein, fat, dietary fibre (mainly chitin) and inorganic matter. The levels of contaminants in the NF depend on the occurrence levels of these substances in the insect feed. Provided that applicable EU legislation regarding feed is followed, the composition of the NF does not raise safety concerns. Regarding the stability of the NF, the Panel notes the limited data provided by the applicant. The Panel cannot conclude on the NF's shelf life since appropriate data for intermediate timespans have not been made available by the applicant. However, 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 Panel could not conclude on the stability of the NF when used as an ingredient in other foodstuffs due to the limitations in the stability data provided by the applicant. The applicant intends to market the NF as an ingredient in several food products. The target population is the general population. Intake was estimated based on the use of the NF as an ingredient in the intended food categories at the maximum proposed levels across surveys in the EFSA Comprehensive European Food Consumption Database. The highest intake estimate was calculated for young children (toddlers), ranging from 60 to 902 mg NF/kg bw per day at the 95th percentile. The Panel notes that consumption of the NF under the proposed uses and use levels does not significantly contribute to the exposure of the population to the analysed undesirable substances, when compared to the rest of the diet. The Panel notes that the NF has 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 limiting amino acids were the sulfur-containing ones. No protein digestibility studies with the NF have been provided. However, the Panel notes that reported protein digestibility values in the literature are variable but are comparable to those of other common foods. 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 affect the bioavailability of minerals. The Panel notes that, taking into account the composition of the NF and the proposed conditions of use, consumption of the NF is not nutritionally disadvantageous. In the light of the fact that no adverse effects were observed in the toxicological studies available in the literature on freeze-dried yellow mealworms, the history of use of the NF and its source, and given that the larvae are reared separately from the adults, the Panel considers that there are no safety concerns.

The Panel considers that the consumption of the NF may induce *de novo* sensitisation and allergic reactions to yellow mealworm proteins and may cause allergic reactions in subjects with allergy to crustaceans and dust mites (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.

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the data claimed as proprietary by the applicant (analyses of contaminants in the NF, detailed description of the drying process, analytical data on chitin levels, and data on the oxidative and microbiological status of the NF during storage).

#### 6. Recommendation

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



## 7. Steps taken by EFSA

- 1) On 03 July 2018 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of dried mealworm (*Tenebrio molitor*) as a novel food Ref. Ares (2018)3529546 - 03/07/2018.
- 2) On 03 July 2018, a valid application on dried mealworm (*Tenebrio molitor*), which was submitted by SAS EAP Group was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2018/0241) and the scientific evaluation procedure was initiated.
- 3) On 20 November 2018, 07 January 2019, 04 April 2019, 28 June 2019, 20 March 2020, 15 October 2020, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
- 4) On 02 January 2019, 22 February 2019, 14 June 2019, 18 March 2020, 11 September 2020, 17 November 2020 additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
- 5) During its meeting on 24 November 2020, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of dried 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
ADME	absorption, distribution, metabolism and excretion
AFNOR	Association Française de Normalisation
AID	apparent ileal digestibility
AMCase	acidic mammalian chitinase
ANFs	antinutritional Factors
AOAC	Association of Official Agricultural Chemists
AV	<i>p</i> -anisidine value
bw	body weight
CONTAM	EFSA Panel on Contaminants in the Food Chain
dl-PCBs	dioxin-like Polychlorinated biphenyls
FAO	Food and Agriculture Organization of the United Nations
fdTML	freeze-dried powdered <i>T. molitor</i> larvae
GC-FID	gas chromatography with flame-ionisation detection
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Points
HPLC	high-performance liquid chromatography
HS-GC/NPD	headspace gas chromatography with nitrogen-phosphorus detector
IC-PAD	ion chromatography-pulsed amperometric detection
ICP-MS	inductively coupled plasma mass spectrometry

IIV-29	invertebrate iridescent virus 29
ISO	International Organization for Standardization
KFDA	Korean Food and Drug Administration
LC-MS/MS	liquid chromatography–tandem mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NF	novel food
OECD	Organisation for Economic Co-operation and Development
OPIE	Office Pour les Insectes et leur Environnement
PCBs	polychlorinated biphenyls
PCEs	polychromatic erythrocytes
PDCAAS	protein digestibility-corrected amino acid score
PV	peroxide value
THCA	1,2,3,4,-tetrahydro-b-carboline-3-carboxylic acid

## Appendix A – Batch-to-batch amino acid analysis

Amino acids (g/100 g NF)	#9	#11	#10	#40	#44
Alanine	3.94	4.33	4.21	4.16	3.92
Aspartic acid	4.82	4.97	4.98	4.93	4.78
Arginine	3.04	3.13	3.09	3.09	2.85
Cystine + Cystein	0.399	0.436	0.394	0.408	0.465
Glycine	2.89	3.09	2.96	3.02	2.98
Glutamic acid	6.68	6.80	6.51	6.54	6.65
Hydroxyproline	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Histidine	1.60	1.71	1.78	1.73	1.66
Isoleucine	2.32	2.46	2.32	2.42	2.43
Leucine	4.08	4.14	4.17	4.18	4.1
Lysine	3.25	3.39	3.33	3.31	3.2
Methionine	0.613	0.637	0.669	0.635	0.695
Ornithine	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Phenylalanine	2.04	2.10	2.21	2.06	2.05
Proline	3.83	3.69	3.54	3.96	4.12
Serine	2.60	2.73	2.76	2.69	2.57
Threonine	2.32	2.43	2.41	2.37	2.24
Tryptophan	0.631	0.661	0.665	0.701	0.675
Tyrosine	3.92	3.89	4.43	4.13	3.82
Valine	3.34	3.49	3.50	3.42	3.35

## Appendix B – Comparison of the amino acid content of the NF to those of other foods

	NF (average)	Barley ( <i>Hordeum vulgare</i> ) whole seed, hulls removed	Fish, fresh, all types (edible portion) <sup>(a)</sup>	Brewer's Yeast <sup>(a)</sup>	Beef and veal ( <i>Bos taurus</i> ) edible flesh <sup>(a)</sup>	Crustaceans (edible portion) <sup>(a)</sup>
<b>Amino acids (g/100 g food)</b>						
<b>Alanine</b>	4.112	0.464	1.126	2.621	1.033	1.073
<b>Arginine</b>	3.040	0.555	1.066	1.944	1.118	1.326
<b>Aspartic acid</b>	4.896	0.665	1.947	4.210	1.590	1.728
<b>Cystine</b>	n.r.	0.267	0.220	0.350	0.230	0.200
<b>Cysteine + Cystine</b>	0.420	n.r.	n.r.	n.r.	n.r.	n.r.
<b>Glutamic acid</b>	6.636	2.771	2.655	4.154	2.703	2.499
<b>Glycine</b>	2.988	0.453	0.906	1.863	0.860	1.044
<b>Histidine*</b>	1.696	0.248	0.665	0.969	0.603	0.300
<b>Hydroxyproline</b>	< 0.05	n.r.	n.r.	n.r.	n.r.	n.r.
<b>Isoleucine*</b>	2.390	0.421	0.900	2.267	0.852	0.745
<b>Leucine*</b>	4.134	0.784	1.445	3.105	1.435	1.388
<b>Lysine*</b>	3.296	0.406	1.713	3.509	1.573	1.262
<b>Methionine*</b>	0.650	0.196	0.539	0.621	0.478	0.466
<b>Ornithine</b>	< 0.05	n.r.	n.r.	n.r.	n.r.	n.r.
<b>Phenylalanine*</b>	2.092	0.603	0.737	1.882	0.778	0.645
<b>Proline</b>	3.828	1.282	0.692	1.497	0.668	0.701
<b>Serine</b>	2.670	0.476	0.816	n.r.	0.713	0.817
<b>Threonine*</b>	2.354	0.389	0.861	2.149	0.812	0.730
<b>Tryptophan*</b>	0.667	n.r.	n.r.	n.r.	n.r.	n.r.
<b>Tyrosine</b>	4.038	0.365	0.689	1.608	0.637	0.581
<b>Valine*</b>	3.420	0.592	1.150	2.850	0.886	0.765

\*: Essential amino acids.

(a): Values from (FAO, 1970).

n.r.: results not reported.



## Appendix C – Batch-to-batch fatty acid analysis

<b>Fatty acids (g/100 g NF)</b>	<b>#1</b>	<b>#2</b>	<b>#3</b>	<b>#4</b>	<b>#5</b>
<b>Total fatty acids, of which</b>	27.16	30.20	21.64	26.42	22.09
<b>Saturated</b>	6.58	7.73	5.19	6.34	5.01
<b>Monounsaturated</b>	13.83	15.37	11.00	12.77	7.06
<b>Polyunsaturated, of which</b>	6.66	7.03	5.41	7.24	10.02
<b>Omega-3</b>	0.26	0.29	0.19	0.27	0.49
<b>Omega-6</b>	6.39	6.73	5.22	6.95	9.47
<b>Omega-6/Omega 3 (ratio)</b>	24.17	23.14	27.15	25.74	19.14
<b>trans</b>	0.09	0.07	0.05	0.07	0.04

## Appendix D – Detailed fatty acid profile analysis of the NF

Fatty acids (% total fatty acids)	#1	#2	#3	#4	#5
<b>Total saturated (SFA)</b>	24.22	25.56	23.94	23.99	22.64
<b>C4:0</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C6:0</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C7:0</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C8:0</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C9:0</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C10:0</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C11:0</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C12:0</b>	0.27	0.27	0.24	0.35	0.04
<b>C13:0</b>	< 0.05	< 0.05	< 0.05	0.07	0.06
<b>C14:0</b>	2.98	2.67	2.93	3.93	2.38
<b>C15:0</b>	0.10	0.10	0.10	0.10	0.24
<b>C16:0</b>	16.95	18.31	16.48	15.53	16.76
<b>C17:0</b>	0.40	0.25	0.38	0.15	0.25
<b>C18:0</b>	3.33	3.81	3.46	3.36	2.67
<b>C19:0</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C20:0</b>	0.13	0.16	0.12	0.12	0.08
<b>C21:0</b>	0.06	< 0.05	< 0.05	< 0.05	< 0.05
<b>C 22:0</b>	< 0.05	< 0.05	0.07	0.08	< 0.05
<b>C24:0</b>	< 0.05	< 0.05	< 0.05	0.31	< 0.05
<b>Total Monounsaturated (MUFA)</b>	50.87	50.83	50.73	48.34	31.92
<b>C11:1</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C12:1</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C13:1</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C14:1 (n-5c)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C 14:1 (n-5t)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C15:1 (n-5c)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C15:1 (n-5t)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C16:1 (n-7c)</b>	1.80	1.68	1.71	1.94	1.05
<b>C16:1 (n-7t)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C17:1 (n-7c)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C17:1 (n-7t)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C18:1 (n-6c)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C18:1 (n-7c)</b>	0.36	0.45	0.47	0.22	0.50
<b>C18:1 (n-7t)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C18:1 (n-9c)</b>	48.59	48.58	48.46	46.08	30.19
<b>C18:1 (n-9t) + C18:1 (n-12t)</b>	0.06	< 0.05	< 0.05	< 0.05	< 0.05
<b>C19:1 (n-12t)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C19:1 (n-9t)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C20:1 (n-9c)</b>	0.12	0.12	0.10	0.09	0.17
<b>C20:1 (n-9t) + C18:2 (10t, 12c) + C20:1 (n-15c)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C22:1 (n-11)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C22:1 (n-9c)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C22:1 (n-9t)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C24:1</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>Total Polyunsaturated (PUFA)</b>	24.49	23.27	24.97	27.40	45.29
<b>C18:2 (9c,11t)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C18:2 (n-6c)</b>	23.52	22.26	24.08	26.08	42.78

<b>Fatty acids (% total fatty acids)</b>	<b>#1</b>	<b>#2</b>	<b>#3</b>	<b>#4</b>	<b>#5</b>
<b>C18:2 (n-6t)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C18:2 t2</b>	0.26	0.24	0.22	0.27	0.18
<b>C18:3 (n-3)</b>	0.97	0.96	0.89	1.03	2.24
<b>C18:3 (n-6)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C18:3 t3 (C18:3 t1 + C18:3 t2)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C18:4 (n-3)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C20:2 (n-6c)</b>	< 0.05	0.05	0.05	< 0.05	0.09
<b>C20:3 (n-3c)</b>	< 0.05	< 0.05	< 0.05	0.06	< 0.05
<b>C20:3 (n-6c)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C20:4 (n-6c)</b>	< 0.05	< 0.05	< 0.05	0.23	< 0.05
<b>C20:5 (n-3c)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C22:2 (n-6c)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C22:5 (n-3c)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C22:5 (n-6c)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>C22:6 (n-3c)</b>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<b>Total Trans</b>	0.32	0.24	0.22	0.27	0.18
<b>Total Omega 3</b>	0.97	0.96	0.89	1.03	2.24
<b>Total Omega 6</b>	23.52	22.26	24.08	26.30	42.78