

SCIENTIFIC OPINION

Scientific Opinion on the safety of refined *Buglossoides* oil as a novel food ingredient¹

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)^{2,3}

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ABSTRACT

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on refined *Buglossoides* oil as a novel food ingredient (NFI) in the context of Regulation (EC) No 258/97. The NFI is produced from the seeds of *Buglossoides arvensis* (L.) I. M. Johnston, using processes conventionally used for edible oil production. The main fatty acids (FAs) contained in the NFI are alpha-linolenic acid (ALA), stearidonic acid (SDA) and linoleic acid, with smaller amounts of oleic acid, gamma-linolenic acid (GLA) and saturated FAs. With the exceptions of SDA and GLA, these FAs are widely present in common foods. The NFI is intended to be used in a range of foods and food supplements to provide approximately 200 mg of SDA per day. Upon digestion, FAs are used primarily as an energy source. ALA and SDA can be elongated and desaturated to produce eicosapentaenoic acid. In human studies using various sources of SDA, no increase or small increases in SDA were observed in blood cell membranes or in total plasma. The proposed specifications for pyrrolizidine alkaloids and erucic acid, which are undesirable substances, do not give rise to concern in view of the proposed conditions of use. The available information does not give concerns as regards other undesirable substances in the NFI. Available animal studies provide only limited information on the safety of the NFI. Human studies that investigated different plant oils or fatty acid ethyl esters as sources of SDA, GLA and ALA found no adverse effects with up to 4 200 mg SDA/day for 12 weeks, up to 1 700 mg GLA/day for 28 days and 9 100 mg ALA/day for four weeks. The Panel concludes that the NFI is safe for the proposed uses and use levels.

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KEY WORDS

Buglossoides oil, stearidonic acid, novel food, ingredient

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on refined *Buglossoides* oil as a novel food ingredient (NFI) in the context of Regulation (EC) No 258/97, taking into account the comments and objections of a scientific nature raised by Member States.

Refined *Buglossoides* oil is a triglyceride oil, in which the polyunsaturated fatty acids (PUFAs) alpha-linolenic acid (ALA, 44 %), stearidonic acid (SDA, 20 %) and linoleic acid (13 %) are the main fatty acids (FAs), with smaller amounts of oleic acid, gamma-linolenic acid (GLA) and saturated FAs. With the exceptions of SDA and GLA, the main FAs present are widely present in common foods. The NFI is produced from seeds of *Buglossoides arvensis* (L.) I. M. Johnst. using processes conventionally used for edible oil production.

With the exceptions of SDA and protein, the content specifications of refined *Buglossoides* oil are similar to those of refined *Echium* oil, which has previously been authorised in the EU as an NFI.

The NFI is intended to be used in a range of foods and in food supplements to provide approximately 200 mg of SDA per day. Based on consumption data of the UK national diet and nutrition survey, the highest mean and 97.5th percentile intakes of SDA, on a per person basis, were calculated for male adults at 1 128 and 2 175 mg/day, respectively, while children had the lowest intake estimates, at 719 and 1 351 mg/day. On a body weight basis, children were calculated to have the highest intake, with mean and 97.5th percentile SDA intakes of 51 and 103 mg/kg body weight, respectively.

Upon digestion, FAs are used primarily as an energy source. ALA and SDA can be elongated and desaturated to produce eicosapentaenoic acid. In human studies using various sources of SDA (750–4 200 mg/day for 3–16 weeks), no increase or small increases in SDA levels were observed in the membranes of blood cells or in total plasma.

Taking into account the composition of the oil and the proposed conditions of use, the Panel considers that consumption of the NFI is not nutritionally disadvantageous.

The microbiological information provided does not give rise to any safety concerns.

The proposed specifications for pyrrolizidine alkaloids and erucic acid, which are undesirable substances, do not give rise to concern in view of the proposed conditions of use. The Panel notes that the available information does not raise concerns as regards other undesirable substances in the NFI.

Three animal feeding studies were carried out with the NFI. No adverse effects were seen in these studies. The Panel notes that these studies were not designed for toxicity testing. A number of animal studies were conducted using SDA- and/or GLA-containing oils from other sources or diets supplemented with SDA ethyl esters. No adverse effects were seen in any of these studies. The Panel notes that most studies were not designed for toxicity testing. The Panel also notes that a number of the animal studies provided were carried out in rodents. The Panel notes that desaturation and elongation of PUFAs are more pronounced in rodents than in humans and therefore extrapolation of results from studies in rodents to humans requires caution. Overall, the Panel considers that the available animal studies provide only limited information on the safety of the NFI.

Human studies that investigated different plant oils or fatty acid ethyl esters as sources of SDA, GLA and ALA found no adverse effects with up to 4 200 mg SDA/day for 12 weeks, up to 1 700 mg GLA/day for 28 days and 9 100 mg ALA/day for four weeks.

The Panel considers that the likelihood of adverse allergic reactions to the NFI is low.

The Panel concludes that the NFI is safe under the proposed uses and use levels.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

On 25 June 2013, the company Technology Crops International submitted a request in accordance with Article 4 of the Novel Food Regulation (EC) No 258/97⁴ to place on the market refined oil from the seeds of *Buglossoides arvensis* as a novel food ingredient (NFI).

On 6 January 2014, the competent authorities of the United Kingdom forwarded to the Commission their initial assessment report, which came to the conclusion that refined oil from the seeds of *Buglossoides arvensis* meets the criteria for acceptance as a novel food defined in Article 3(1) of Regulation (EC) No 258/97.

On 21 January 2014, the Commission forwarded the initial assessment report to the other Member States. Several of the Member States submitted comments or raised objections.

In consequence, a decision is now required by the Commission under Article 7(1) of Regulation (EC) No 258/97.

The concerns of a scientific nature raised by the Member States can be summarised as follows:

- The analysis of polycyclic aromatic hydrocarbons (PAHs) was carried out using a method stipulating a detection limit of 2 µg/kg for benzo(a)pyrene. This method does not appear to be suitable for verifying compliance with the maximum permissible level of 2 µg/kg of benzo(a)pyrene for oils, as stipulated in Regulation (EC) No 1881/2006.⁵ In order to verify compliance, the *limit of quantification* of the method used should be at least 2 µg/kg.
- Whether the testing facilities are accredited in accordance with an international system is not apparent from the documentation.
- It was noted that the safety of the novel oil was fully based on the compositional resemblance to the authorised refined *Echium* oil which is derived from seeds of *Echium Plantagineum*. Although *Buglossoides arvensis* is taxonomically related to *Echium Plantagineum*, as both plants belong to the same *Boraginaceae* family, these are distinct species.
- *Buglossoides arvensis* (L.) contains active substances such as pyrrolizidine alkaloids and has been described as having *in vitro* antigonadotropic activity.
- With respect to the stability of the oil, studies were performed for only eight weeks at a maximum temperature of 60 °C, in darkness, in sealed vials and using nitrogen. In the event that these precautions against oxidation are not taken under the conditions of use of the oil, a study should be performed under the real storage conditions during the average expected life of the product.
- During the processing of the foods for which the novel ingredient is intended, the temperatures reached can cause changes to the properties of the oil, which should also be investigated.
- Some results of *in vivo* studies in mice and salmon, performed using oil from *Buglossoides*, are provided. From the information available, it is not possible to know whether good laboratory practice (GLP) or standardised (OECD) protocols have been followed. Only females were used in the research on mice, and there was no information on important indicators (consumption of food and water, haematology, clinical biochemistry, histopathology, etc.).

⁴ Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L 43, 14.2.1997, p. 1–6.

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

- Safety studies on individual components obtained from various sources or similar oils are also provided. In the case of gamma-linolenic acid, it is noted that the effect on the growth of the test animals differs according to the origin of the component.
- None of the studies on humans were performed using the novel food ingredient, but rather using one of its components obtained from other sources or similar oils.
- No toxicological information from studies in experimental animals nor data from investigations in humans were provided. The presence of possible harmful substances cannot be excluded based on the fact that the novel oil is highly purified, but requires additional safety studies.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002,⁶ the European Commission asks the European Food Safety Authority to provide a scientific opinion by carrying out the additional assessment for refined oil from the seeds of *Buglossoides arvensis* as a novel food ingredient in the context of Regulation (EC) No 258/97.

⁶ Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

ASSESSMENT

In accordance with Commission Recommendation 97/618/EC,⁷ refined oil from the seeds of *Buglossoides arvensis* (hereafter called refined *Buglossoides* oil) is allocated to Class 2.2, i.e. “complex NF from non-GM source. The source of the NF has no history of food use in the Community”. The assessment of the safety of this novel food ingredient (NFI) is based on data supplied in the original application, the initial assessment by the competent authority of the United Kingdom, the concerns and objections of the other Member States and the responses of the applicant. The data are required to comply with the information required for novel foods of Class 2.2, i.e. structured schemes I, II, III, IX, XI, XII and XIII of Commission Recommendation 97/618/EC. These structured schemes are addressed in Sections 1 to 7. The intention is to market refined *Buglossoides* oil for use in a range of foods and food supplements as a source of n-3 and n-6 polyunsaturated fatty acids (PUFAs). This assessment concerns only risks that might be associated with consumption, and is not an assessment of the efficacy of refined *Buglossoides* oil with regard to any claimed benefit.

1. Specification of the NFI

Buglossoides oil is a refined vegetable oil obtained from the seeds of *Buglossoides arvensis* (L.) I. M. Johnst.⁸ The specifications proposed by the applicant are presented in Table 1. Except for the content of the fatty acid stearidonic acid (SDA, C18:4(n-3)) and the protein content, the parameters and limits included in the specifications are the same as those set for “refined *Echium* oil”, an oil extracted from *Echium plantagineum* and previously authorised in the EU as a novel food ingredient in 2008.⁹ The applicant noted that *Buglossoides arvensis* is taxonomically related to *Echium plantagineum*, both being members of the *Boraginoideae* subfamily within the *Boraginaceae* family. According to the applicant, the taxonomic difference between the tribe containing *Buglossoides* (*Lithospermeae*) and the tribe containing *Echium* (*Echieae*) mainly relates to “their zygomorphic corolla with the stamens inserted at different heights”.

Table 1: Specifications of refined *Buglossoides* oil proposed by the applicant

	Specification
Description of <i>Buglossoides</i> oil	The pale yellow product obtained by refining oil extracted from the seeds of <i>Buglossoides arvensis</i> (L.) I. M. Johnst.
Stearidonic acid	Not less than 15 % w/w of total fatty acids
<i>Trans</i> fatty acids	Not more than 2 % w/w of total fatty acids
Acid value	Not more than 0.6 mg KOH/g
Peroxide value	Not more than 5 meq O ₂ /kg
Unsaponifiable content	Not more than 2 %
Protein content (total nitrogen)	Not more than 10 µg/g
Pyrrrolizidine alkaloids	Not detectable with a detection limit of 4 µg/kg

The applicant provided analytical data from three non-consecutive batches of the NFI (Table 2). Batches NZ00053 and NZ00056 were produced using the cold press process and batch NZ00058 by extraction using hexane as the solvent. The three batches complied with the specifications.

⁷ Commission Recommendation 97/618/EC: Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council. OJ L 253, 16.9.1997, p. 1–36.

⁸ Taxonomic classification: Kingdom: *Plantae*; Phylum: *Magnoliophyta*; Class: *Magnoliopsida*; Order: *Boraginales*; Family: *Boraginaceae*; Genus: *Buglossoides*; Species: *Buglossoides arvensis* (L.) I. M. Johnst.

⁹ Commission Decision of 27 June 2008 authorising the placing on the market of refined echium oil as novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council (2008/558 EC). OJ L 180, 9.7.2008, p. 17–19.

Table 2: Analytical data from three batches of refined *Buglossoides* oil

	Batch			Method
	NZ00053	NZ00056	NZ00058	
Stearidonic acid (% w/w of total fatty acids)	20.5	19.7	20.8	AOCS Ce 1h-05
<i>Trans</i> fatty acids (% w/w/ of total fatty acids)	< 1.0	< 1.0	< 1.0	AOCS Ce 1h-05
Acid value (mg KOH/g)	0.22	0.12	0.34	AOCS Ca 5a-40
Peroxide value (meq O ₂ /kg)	2.03	1.55	1.22	AOCS Cd 8-53
Unsaponifiable content (%)	0.28	0.43	0.73	AOCS Ca 6a-40
Protein content (total nitrogen) (µg/g)	1.3	1.0	1.3	Rigby et al. (2011)
Pyrrolizidine alkaloids (µg/kg)	< 1.0	< 1.0	< 1.0	Cramer et al. (2013)

AOCS, American Oil Chemists' Society.

Buglossoides oil consists primarily of triglycerides (90 %) with small amounts of di- and monoglycerides, free fatty acids (2–6 %, 2–4 % and < 0.3 % respectively), and an unsaponifiable fraction (< 2 %). The unsaponifiable fraction comprises phytosterols, principally β-sitosterol, campesterol and stigmasterol, and γ-tocopherol.

Alpha-linolenic acid (ALA), stearidonic acid (SDA) and linoleic acid (LA) are the major fatty acids in the oil. The fatty acid compositions of three batches of the NFI are provided in Table 3; they cover more than 98 % of the fatty acids present in the oil.

Table 3: Batch analysis of fatty acid composition of *Buglossoides* oil

	% Composition of total fatty acids ^(a)			
	NZ00053	NZ00056	NZ00058	Mean
Alpha-linolenic acid (C18:3(n-3))	44	44	43.5	43.8
Stearidonic acid (C18:4(n-3))	20.5	19.7	20.8	20.3
Linoleic acid (C18:2(n-6))	12.7	12.7	12.7	12.7
Oleic acid (C18:1(n-9))	7.6	7.6	7.5	7.6
Gamma-linolenic acid (C18:3(n-6))	6.4	6.2	6.3	6.3
Palmitic acid (C16:0)	5.2	5.3	5.2	5.2
Stearic acid (C18:0)	1.8	1.9	1.8	1.8
Gondoic acid (C20:1(n-9))	0.7	0.8	0.8	0.8
Erucic acid (C22:1(n-9))	0.2	0.2	0.2	0.2
Lignoceric acid (C24:0)	< 0.1	< 0.1	< 0.1	< 0.1
Palmitoleic acid (C16:1(n-7))	0.1	0	0.1	0.1
Nervonic acid (C24:1(n-9))	0.1	0	0	0

(a): Gas–liquid chromatography analysis, AOCS Ce 1h-05.

The applicant noted that the composition of the NFI is similar to the composition of refined *Echium* oil, with the exception that the relative ratios of SDA and ALA are higher in the NFI.

The NFI was analysed for protein content using the Bradford method and the combustion/chemiluminescence method. The protein contents in the three batches were below the limits of detection (10 ppm). Considering the limitations of the Bradford method for confirming the absence of protein (ACNFP, 2011), and the limited sensitivity of the combustion/chemiluminescence method, further analyses were performed using a method based on extraction using a borate buffer and protein quantification using 3-(4-carboxybenzoyl)quinolone-2-carboxaldehyde (Rigby et al., 2011). The results for the three batches were in the range 1.0–1.3 µg/g, which is below the maximum protein content of 10 µg/g set in the specifications of the NFI.

Trans fatty acids were not detected in any of the three batches analysed (limit of detection (LOD): 1 %). A maximum limit of 2 % is set in the specifications.

According to the specifications, the maximum peroxide value is 5 milliequivalent (meq) O₂/kg which is below the maximum limit of up to 10 meq O₂/kg oil for refined oils proposed by Codex Alimentarius (1999). Epoxy fatty acids were not detected in any of the three batches analysed (LOD: 0.1 %). The applicant also provided the results of a p-anisidine test as a stability parameter.

According to the specifications, the maximum acid value is 0.6 mg KOH/g which is the maximum limit for refined oils proposed by Codex Alimentarius (1999).

Erucic acid is found in the NFI at levels of around 0.2 %, which is below the maximum level of 5 % in oils and fats intended for human consumption set by EU legislation.¹⁰

Buglossoides arvensis synthesises pyrrolizidine alkaloids (PAs). The applicant reports that a sample of unrefined *Buglossoides* oil was found to contain a total of 44 µg/kg PAs, but refining reduced this level to below 1 µg/kg. A maximum limit of 4 µg/kg is set in the specification. The Panel on Contaminants in the Food Chain has evaluated the risks to human health related to the presence of PAs in food and concluded that 1,2-unsaturated PAs may act as genotoxic carcinogens in humans (EFSA CONTAM Panel, 2011). A benchmark dose lower confidence limit for a 10 % excess cancer risk of 70 µg/kg body weight per day for the induction of liver haemangiosarcomas by lasiocarpine in male rats was calculated as the reference point for comparison with the estimated dietary exposure. Assuming a maximum PA content of 4 µg/kg in refined *Buglossoides* oil and an intake at the 97.5th percentile of 103 mg SDA/kg body weight per day, which is equivalent to 515 mg NFI/kg body weight per day, a child would be exposed to 2.1×10^{-3} µg PAs/kg body weight per day, which corresponds to a margin of exposure (MOE) of 34 000.

The data on PA content, provided in Table 1 as part of the original application, were elaborated by a university research laboratory which specialises in this methodology. The applicant also provided data on additional analyses performed by the National Research Council Canada. The analysis of four batches, including NZ00056 and NZ00058, confirmed that traces of PAs were present at or above the limit of detection of 0.14 µg retronecine equivalents/kg; however, the contents were below the limit of quantification of 0.47 µg retronecine equivalents/kg. A full description of the analysis and the validation has been provided.

The method was also used to investigate samples of crude and refined *Buglossoides* oils from different regions (UK, New Zealand and Canada). There were differences in the amounts of PAs in the crude oils (average 4.6–14.4 µg retronecine equivalents/kg); however, by comparing the content of the refined oil samples and the crude oil used as a source, a refining process including deodorisation and alkali wash was estimated to result in an approximately 25-fold reduction of the PA content. According to the applicant, commercial batches of *Buglossoides* oil will be subjected to at least one acid wash in addition to the other refining steps, as this has been demonstrated to result in further reductions in the PA content of between 32- and 1000-fold (Cramer et al., 2014).

Finally, the applicant provided data on the analysis of individual PAs in *Buglossoides* oil performed in an accredited laboratory. None of 18 specific PAs was found (limit of quantification: 1 µg/kg) in cold pressed or extracted crude *Buglossoides* oils.

Pesticide residues were not detected in analyses of the three batches of the NFI.

The applicant provided analytical results of the three batches for heavy metals, dioxin and dioxin-like polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs), which were below

¹⁰ Council Directive 76/621/EEC of 20 July 1976 relating to the fixing of the maximum level of erucic acid in oils and fats intended as such for human consumption and in foodstuffs containing added oils or fats. OJ L 202, 28.7.1976, p. 1–4.

the maximum levels for contaminants in fats and oils set by EU legislation.¹¹ Upon request from a Member State, the applicant contacted the laboratory which carried out the analysis for benzo(a)pyrene. It clarified that the limit of quantification was 2.0 µg/kg and that the detection limit was 0.6 µg/kg. The results of the three batches were below the limit of quantification.

All analyses were carried out by laboratories which hold internationally recognised accreditations.

The Panel considers that the information provided on the composition, the specification and the batch-to-batch variability of the NFI is sufficient and does not raise safety concerns.

1.1. Stability

The applicant indicated that authorised antioxidants may be added to the NFI, at levels compliant with EU legislation on food additives.

In a stability study, samples of the three production batches were placed in sealed air-tight glass vials with nitrogen gas in the headspace, with or without the addition of 1 000 ppm of natural mixed tocopherols containing a minimum of 700 mg/g of d-γ-tocopherol as an antioxidant. Vials were stored at either 4 °C, 22 °C or 60 °C in the dark. After eight weeks, the peroxide values of all samples were found to be within specification. In response to a comment from a Member State, results after 12 months were also provided by the applicant, which were compliant with the specification.

According to the applicant, the storage condition of sealed vials purged with nitrogen closely mimics the storage conditions for bulk oil after manufacture and is a good model for oil packed in softgel capsules and for oil supplied in sealed bottles. For other uses as a food ingredient, the applicant indicates that the stability would have to be assessed individually for each product, as it would be affected by the particular combination of recipe, processing and packaging used and the presence of antioxidants.

The Panel considers that these data provide sufficient information with respect to the stability of the NFI.

2. Effect of the production process applied to the NFI

The NFI is produced from the seeds of *Buglossoides arvensis* (L.) I. M. Johnst. Seeds are tested for purity, moisture and crop identity prior to manufacture.

The oil may be extracted by expeller pressing or solvent extraction.

In the first method, seeds are crushed or flaked and then passed through a screw press expeller. The temperature of the oil during the isolation step is normally in the range of 50–85 °C. The oil is collected, filtered and transferred to storage containers under nitrogen or another inert gas. The meal remaining after expeller pressing contains residual oil and is commonly re-extracted using solvent extraction.

In the second method, seeds are lightly crushed or flaked and then immersed under nitrogen in an organic solvent (normally hexane or isohexane) at approximately 60 °C, which extracts the oil into solution. The oil/solvent mixture is decanted off, filtered and the solvent removed by evaporation followed by vacuum steam stripping. The oil is then transferred to storage containers.

After extraction, crude *Buglossoides* oil is refined by methods commonly used in the edible oil industry, such as degumming, addition of sodium hydroxide to neutralise free fatty acids, bleaching, deodorisation and filtration. According to the applicant, commercial batches of *Buglossoides* oil will

¹¹ 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.

be subjected to at least one acid wash in addition to the other refining steps, which has been demonstrated to result in a substantial reduction in the content of PAs (see Section 1).

The finished oil is transferred to containers and the headspace is flushed with nitrogen prior to being sealed.

Processing aids and food additives employed in the manufacture of the NFI are described in the application dossier. They meet food-grade specifications and are used in compliance with appropriate EU regulations.

Manufacture is in conformance with current good manufacturing practices and with ISO 22000:2005, which incorporates the Hazard Analysis and Critical Control Points system.

Once processing is complete, the oil is tested against the product specifications. The applicant also indicates that a sampling system for random batches of oil will be employed to monitor levels of undesirable substances such as PCBs, dioxins and pesticides.

The applicant notes that any use of the oil will require the manufacturer to protect against exposure to air, especially if the oil is exposed to high temperatures, and the oxidation status of the oil will be tested in such cases to ensure that degradation has not taken place. For this reason, it will be recommended that the NFI should not be used for high-temperature cooking in the home (e.g. frying).

A Member State requested information on the potential effects of high temperature on the properties of the oil. According to the applicant, changes in the configuration of the double bonds from *cis* to *trans* are negligible at temperatures below 220 °C. The Panel notes that some conjugated double bonds might be formed at these temperatures. The applicant indicates that processing temperatures are kept below this value.

The manufacture of the NFI is based on procedures which are commonly employed in the production of edible oils. The Panel considers that the production process is sufficiently described and does not raise safety concerns.

3. History of the organism used as a source

Buglossoides arvensis was first described and classified as *Lithospermum arvense* by Linnaeus (1753). It has been described more recently by Clapham et al. (1962). The plant is native to the UK and is found in many parts of Europe and North America. The taxonomy of *Buglossoides arvensis* is described in Section 1.

The applicant noted that *Buglossoides* oil has no history of use in food and, therefore, is not normally present in the diet. Other uses of the source are reported in Section 7.4.

4. Anticipated intake/extent of use of the NFI

The applicant intends to market the NFI for use in a range of foods and food supplements. The applicant's aim is to provide approximately 200 mg of SDA per day.

The applicant proposes that the NFI should be used in the same foods as those in which *Echium* oil is authorised, and in such proportions as to give the same maximum levels of SDA as those approved for *Echium* oil. The proposed uses and maximum levels of SDA are presented in Table 4.

Table 4: Intended uses of refined *Buglossoides* oil

Use group	Maximum level of stearidonic acid
Milk-based products and drinkable yoghurt products delivered in a single dose	250 mg/100 g; 75 mg/100 g for drinks
Cheese preparations	750 mg/100 g
Spreadable fat and dressings	750 mg/100 g
Breakfast cereals	625 mg/100 g
Food supplements	500 mg/day as recommended by the manufacturer
Dietary foods for special medical purposes	in accordance with the particular nutritional requirements of the persons for whom the products are intended
Foods intended for use in energy-restricted diets for weight reduction	250 mg/meal replacement

The applicant refers to the estimated intake of SDA from *Echium* oil which was provided in the application for the approval of refined *Echium* oil as an NFI (Croda Chemicals Europe Ltd., 2006) as a basis for estimating the intake of the NFI. Estimates were derived from consumption data of the UK national diet and nutrition survey (NDNS).

Among the population groups, on a per person basis, the highest mean and 97.5th percentile intakes of SDA of 1 128 and 2 175 mg/day, respectively, were calculated for male adults, while children had the lowest intake estimates at 719 and 1 351 mg/day (Table 5), respectively.

Table 5: Summary of the estimated daily intakes of SDA (NDNS data)

Population group	Age group (years)	Users (%)	Users (n)	All person consumption (mg/day)				All user consumption (mg/day)			
				Mean	P90	P95	P97.5	Mean	P90	P95	P97.5
Children	1.5–4.5	98.8	1 628	719	1 053	1 216	1 354	719	1 053	1 208	1 351
Young people	4–10	99.6	834	860	1 234	1 371	1 561	860	1 245	1 374	1 561
Female teenagers	11–18	97.8	436	805	1 265	1 403	1 594	804	1 271	1 418	1 594
Male teenagers	11–18	99.5	414	1 056	1 647	1 873	2 076	1 057	1 647	1 873	2 091
Female adults	16–64	94.3	903	866	1 325	1 507	1 692	871	1 326	1 520	1 693
Male adults	16–64	95.0	728	1 124	1 751	1 932	2 189	1 128	1 752	1 928	2 175

P90, 90th percentile; P95, 95th percentile; P97.5, 97.5th percentile.

On a body weight basis, children were calculated to have the highest intakes, at both the mean and 97.5th percentile, with SDA intakes of 51 and 103 mg/kg body weight, respectively (Table 6).

Table 6: Summary of the estimated daily intakes of SDA per kg body weight (NDNS data)

Population group	Age group (years)	Users (%)	Users (n)	All person consumption (mg/kg body weight/day)				All user consumption (mg/kg body weight/day)			
				Mean	P90	P95	P97.5	Mean	P90	P95	P97.5
Children	1.5–4.5	98.8	1 628	50	76	86	103	51	76	86	103
Young people	4–10	99.6	834	34	51	58	68	34	51	58	68
Female teenagers	11–18	97.8	436	15	25	28	32	15	25	28	32
Male teenagers	11–18	99.5	414	20	32	35	39	20	32	35	39
Female adults	16–64	94.3	903	12	20	23	26	13	20	23	26
Male adults	16–64	95.0	728	13	22	24	28	14	22	25	28

P90, 90th percentile; P95, 95th percentile; P97.5, 97.5th percentile.

The applicant notes that the estimated intakes represent an overestimate of the consumption of SDA from *Buglossoides* oil, because the estimates were based on a larger number of food groups than those which were included in the final authorisation for refined *Echium* oil and considered in the present application.

The applicant also notes that both SDA and ALA levels will be comparable in foods containing the NFI and in foods containing *Echium* oil, but the added quantity of NFI will be lower as it contains higher concentrations of both fatty acids.

5. Nutritional information on the NFI

The applicant intends that the NFI will primarily be a replacement for *Echium* oil. The NFI has a higher proportion of SDA, so that less oil is required to provide the same intake of SDA.

The main fatty acids contained in the NFI are the PUFAs ALA (44 % of the total fatty acid (TFA) content), SDA (20 %) and LA (13 %), respectively, with smaller amounts of oleic acid and gamma-linolenic acid (GLA), as described in Section 1. The saturated fatty acids palmitic acid and stearic acid represent 5 % and 2 % of the TFA content, respectively. *Trans* fatty acids represent less than 2 % of the TFA content, as per the NFI specifications.

SDA can act as a precursor for eicosapentaenoic acid (EPA) (Section 7.1.1). With the exceptions of SDA and GLA, the main fatty acids present in the NFI are widely present in common foods.

In its opinion on Dietary Reference Values for fats, the Panel set an Adequate Intake (AI) of 4 E % for LA and an AI of 0.5 E % for ALA (EFSA NDA Panel, 2010). No Tolerable Upper Intake Levels were set for those fatty acids. In view of the continuous relationship between saturated and *trans* fatty acid intake and increases in blood cholesterol concentrations, the Panel concluded that saturated and *trans* fatty acid intake should be as low as possible within the context of a nutritionally adequate diet.

The intended use level of the NFI is 200 mg of SDA per day (Section 4), which would correspond to approximately 985 mg total fat, 430 mg ALA and 125 mg LA per day.

At the proposed use level, the intake of palmitic acid and stearic acid would amount to approximately 50 mg and 20 mg, respectively. At the maximum content of 2 %, this would correspond to an intake of 20 mg *trans* fatty acids. These amounts are low compared with observed intakes of these fatty acids in European countries (EFSA NDA Panel, 2010) and are not considered a concern.

Taking into account the composition of the oil and the proposed conditions of use, the Panel considers that consumption of the NFI is not nutritionally disadvantageous.

6. Microbiological information on the NFI

The processes used to extract and to refine the NFI include temperatures in excess of 90 °C under vacuum for tens of minutes, and filtration at the micron level. The oil itself has a very low water content and activity. The applicant has presented microbiological analyses confirming the absence of microbial contamination (yeasts, moulds, *Enterobacteria* and *S. aureus*) in three separate batches of the NFI.

The Panel considers that the microbiological information provided does not raise safety concerns.

7. Toxicological information on the NFI

7.1. Absorption, distribution, metabolism and excretion

The component fatty acids of the NFI are released from the glycerides upon digestion and are used primarily as an energy source. The fatty acids can also be metabolised to longer chain or more unsaturated fatty acids. ALA and SDA can be elongated and desaturated to produce EPA.

Some studies have investigated the metabolism of SDA in rodents (Huang et al., 1991; Yamazaki et al., 1992). The Panel notes that the ability to desaturate and elongate precursors of PUFAs is more pronounced in rodents than in humans and therefore extrapolation of results from studies in rodents to humans requires caution (Whelan, 2009; EFSA GMO Panel, 2014).

In human subjects, the efficiency of conversion to EPA is greater with SDA than with ALA, as indicated by significantly higher increases in EPA concentrations in erythrocytes and plasma phospholipids obtained after SDA intake than after ALA intake (James et al., 2003). Efficiencies of conversion of SDA to EPA of between 3:1 and 6:1 have been reported, as measured by changes in the percentage of EPA in erythrocytes (James et al., 2003; Harris et al., 2008; Lemke et al., 2010).

In human studies in which SDA (750–4 200 mg/day for 3–16 weeks) was administered in the form of SDA ethyl esters (James et al., 2003), *Echium* oil (Miles et al., 2004b; Surette et al., 2004) or SDA-enriched soybean oil (Harris et al., 2008; Lemke et al., 2010), no increase or only small increases in SDA were observed in the membranes of blood cells (erythrocytes, neutrophils and granulocytes) or in total plasma.

7.2. Toxicity studies

7.2.1. Studies with the NFI

Three animal studies were carried out with the NFI.

This included two mouse feeding studies, which aimed to investigate the effect of the NFI alone or in combination with a peptide preparation from hydrolysed milk protein on immunity parameters, mammary tumour development and the fatty acid composition of tissues (Surette and Matar, 2012; Surette, 2013). In the first period of both studies, mice received a control diet or a diet containing the NFI so that 1 % of energy was supplied as SDA (equivalent to 3 900 mg NFI/kg body weight per day and 780 mg SDA/kg body weight per day) for 28 and 56 days, respectively. In the second period, mice received the peptide preparation or a saline solution for (cycles of) seven days, with (study 2) or without (study 1) a challenge with mammary carcinoma cells. They were then sacrificed and tissues were processed for the various analyses. During the course of the studies, mice were inspected daily for “general health status”: respiration, colour of paws, muscle tone and signs of distress and dehydration. This inspection did not reveal any adverse effects in these two studies.

In the third study, Atlantic salmon (*Salmo salar* L.) fry received a diet containing the NFI at a level of 11.5 % or a control diet (containing herring oil) for 56 days (unpublished study report by Plante and Surette (2012)). At the end of this period, fish condition, specific growth rate, mortality, per cent lipid deposited (plus fatty acid analysis) and gross energy content were assessed. No adverse effects were reported.

The Panel considers that these studies were not designed for toxicity testing and thus provide only limited information on the safety of the NFI.

7.2.2. Studies with other oils

The applicant referred to a number of studies which investigated the effect of SDA- and/or GLA-rich oils from other sources or diets supplemented with SDA ethyl esters, in various species.

7.2.2.1. Toxicity studies

A 28-day gavage study and a sub-chronic 90-day feeding study combined with a one-generation reproduction toxicity study were conducted in rats using SDA-rich soybean oil (SDA content of 20 % and 26%, respectively), compliant with OECD guidelines (Hammond et al., 2008). No adverse effects were observed in these studies and the no-observed-adverse-effect-levels were 3 mL SDA-rich soybean oil/kg body weight per day (corresponding to approximately 600 mg SDA/kg body weight per day) and 4 000 mg SDA-rich soybean oil/kg body weight per day (corresponding to approximately

1 000 mg SDA/kg body weight per day), the highest doses tested in the respective studies (EFSA GMO Panel, 2014).

Two longer-term toxicity studies with evening primrose oil in rats (dose of up to 2.5 mL evening primrose oil/kg body weight, corresponding to 230 mg GLA/kg body weight per day for 53 weeks) and beagle dogs (dose of up to 5 mL evening primrose oil/kg body weight, corresponding to 450 mg GLA/kg body weight per day for 52 weeks) did not reveal any adverse effects (Everett et al., 1988).

The Panel notes that at the highest dose tested (corresponding to 1 000 mg SDA/kg body weight per day) SDA-rich soybean oil showed no adverse effects in a 90-day/one-generation reproduction feeding study in rats. Similarly, the highest dose of evening primrose oil tested in a 53-week toxicity study in rats (corresponding to 230 mg GLA/kg body weight per day) and a 52-week toxicity study in beagle dogs (corresponding to 450 mg GLA/kg body weight per day) did not show any adverse effects.

7.2.2.2. Other feeding studies

Other studies were conducted in rats (doses between 130 mg SDA/kg body weight per day for three months and 1 000 mg SDA ethyl esters/kg body weight per day for three weeks) (Yamazaki et al., 1992; Engler, 1993; Barzanti et al., 1995), mice (2 000 mg SDA/kg body weight for three weeks) (Ishihara et al., 2002), guinea pigs (100 mg SDA/kg body weight per day for 40 days) (Crozier et al., 1989) and beagle dogs (up to 193 mg SDA/kg body weight for 12 weeks) (Harris et al., 2007). Endpoints examined differed amongst studies and included food consumption, changes in body and liver weights, blood pressure, serum lipid concentrations, fatty acid composition of blood cells and tissues (liver and heart) and concentrations of inflammatory mediators. No adverse effects were reported.

One study of rats which received various doses of GLA in the form of evening primrose oil, borage oil, blackcurrant oil and fungal oil (880, 2 090, 1 870, 2 860 mg GLA/kg body weight per day, respectively) or a control diet (sesame oil) did not find any significant effects of any treatment on body weight (Engler, 1993). A significant increase in serum cholesterol in the borage oil group compared with the sesame oil and fungal oil groups was reported. In a study of female mice, Wainwright et al. (2003) administered borage oil (containing 23 % GLA) or two types of genetically modified rapeseed oil (containing 23 or 36 % GLA, respectively) at doses corresponding to 3 400 to 5 400 mg GLA/kg body weight per day for six months. No treatment related-effects were found on reproductive-related outcomes. Reduced pup body weight associated with a slight increase in neonatal pup attrition was found in the groups receiving the rapeseed oils. There were no significant effects on pup behavioural development or on performance in plus maze tests.

The Panel notes that animal feeding studies using oils from different plant sources of various fatty acid compositions as sources of SDA and/or GLA did not indicate any adverse effects at levels of up to 2 000 mg SDA/kg body weight for three weeks and up to 5 400 mg GLA/kg body weight for six months in mice. These studies were not designed for toxicity testing and thus provide limited information on the safety of the NFI.

7.3. Human studies

The applicant referred to studies conducted in humans which investigated SDA, GLA and ALA. Sources used were SDA ethyl esters, SDA-rich soybean oil and *Echium* oil (containing a combination of SDA and GLA), borage oil (rich in GLA), and flaxseed oil or a blended oil (rich in ALA).

One study found no effects of 1 500 mg SDA/day for six weeks, administered as SDA ethyl esters, on coagulation parameters, concentrations of immune markers and concentrations of blood lipids compared with similar doses of ALA or EPA ethyl esters (James et al., 2003).

With respect to SDA- and GLA-rich oils, no adverse effects were found on the concentrations of blood lipids, blood pressure, heart rate, platelet function, standard blood laboratory tests and adverse events

recording in studies using SDA-rich soybean oil (doses of 3 700 mg SDA/day plus 1 200 mg GLA/day for 16 weeks and 4 200 mg SDA/day plus 1 100 mg GLA/day for 12 weeks) (Harris et al., 2008; Lemke et al., 2010) or *Echium* oil (doses of 1 900 mg SDA/day plus 1 700 mg GLA/day for 28 days and 1 000 mg SDA/day plus 900 mg GLA/day for 12 weeks) (Miles et al., 2004b; Miles et al., 2004a; Surette et al., 2004; Miles et al., 2006).

Two studies which used ALA-rich flaxseed oil (dose of 20 000 mg ALA/day for 56 days and 6 600 mg ALA/day for 16 weeks) or blended oil (9 100 mg ALA/day for four weeks) did not report any adverse effects on concentrations of blood lipids, coagulation parameters, blood pressure, heart rate, standard blood laboratory tests, urinalysis, or adverse events recording (Kelley et al., 1993; Takeuchi et al., 2007; Gracious et al., 2010).

The Panel notes that human studies using oils from different plant sources of various fatty acid compositions as sources of SDA, GLA and/or ALA (up to 4 200 mg SDA/day for 12 weeks, up to 1 700 mg GLA/day for 28 days and up to 9 100 mg ALA/day for four weeks) did not show any adverse effects.

7.4. Possible presence of substances of concern

Information on erucic acid and PAs is provided in Section 1.

The applicant carried out a literature search in order to identify other possible undesirable substances in *Buglossoides arvensis* and related species.

One *in vitro* study investigated the antigonadotropic activity of an aqueous extract from the roots of *Lithospermum ruderale*, a related species, on bovine steroid receptors (Findley and Jacobs, 1980). High concentrations of the extract (3.0 mg/mL uterine cytosol preparation) were required to displace progesterone, testosterone and oestradiol from their respective uterine receptors. Because of the high dose required and the different species, part of the plant and type of extract used, the Panel does not consider this relevant for the safety evaluation of the NFI.

One review mentioned androgenic, gonadotropic and oestrogenic effects of the leaves and seeds of *Lithospermum arvense* [*Buglossoides arvensis*] (Sandroni, 2001). This review refers to a study in which a hot water extract of plant seeds was administered orally to rats (Ilarionov, 1989). The Panel considers that this report does not give rise to safety concerns regarding the NFI because the study was conducted with an aqueous extract. Water-soluble compounds are expected to partition into the aqueous phase during the extraction of the oil and to be largely removed from the oil in the course of the subsequent refining.

One study investigated the distribution in plants of phytoecdysteroids, which are plant-produced analogues of steroidal insect hormones; the presence of these compounds was reported in the seeds and leaves of *Buglossoides arvensis* (Dinan et al., 2001). The Panel notes that phytoecdysteroids are polar molecules and considers that they would partition into the aqueous fractions during extraction and refining and are unlikely to be present in the refined oil.

One review reported the presence of naphthoquinones (acetylshikonin, isobutyrylshikonin, isovalerylshikonin and beta-hydroxyisovalerylshikonin) in the roots of *Lithospermum arvense* [*Buglossoides arvensis*] (Papageorgiou et al., 1999). Shikonin and several related naphthoquinones have also been identified in the roots of *Echium plantagineum* (Weston et al., 2012). Shikonin derivatives extracted from the roots of *Arnebia euchroma* (Royle) Johnst. (Boraginaceae) were administered by gavage to Wistar rats at concentrations of 200, 400 and 800 mg/kg body weight per day for 90 days or 180 days (Su et al., 2013). Haematological and biochemical examinations were performed, and the vital organs were subjected to pathological analyses. No toxicity was observed.

The applicant noted that shikonin biosynthesis is inhibited by light (Brigham et al., 1999; Yazaki et al., 2001) and that shikonin has been found only in roots of *Buglossoides arvensis* (Papageorgiou et

al., 1999). Considering that the oil is exclusively extracted from the seeds of *Buglossoides arvensis*, the Panel considers that naphthoquinones present in the roots of *Buglossoides arvensis* are not relevant for the safety assessment of the NFI.

The applicant referred to the EFSA scientific cooperation (ESCO) report on a compendium of botanical species that have been reported to contain toxic, addictive, psychotropic or other substances of concern (EFSA, 2009). No entries relate to *Buglossoides* species. There are two entries relating to *Lithospermum* spp., which report the presence of PAs. No other substances of concern have been reported in any species of the *Boraginaceae* family. Several species have been traditionally used as foodstuffs or medicines (for instance, *Borago officinalis* (seed oil, flowers and leaf tea), *Echium plantagineum* (seed oil) and *Symphytum officinale* (leaf or root tea)). The Panel notes that an update of this compendium was published in 2012 (EFSA, 2012). No additional information of relevance for this assessment is provided in this update.

The Panel notes that the available information does not raise concerns as regards undesirable substances in the NFI other than PAs and erucic acid.

8. Allergenicity

The applicant states that no allergic reactions to *Buglossoides arvensis* have been reported. No allergens of *Buglossoides arvensis* have been identified in the literature or in the Allergome database.¹²

The Panel notes that *Buglossoides* oil is refined by methods commonly used in the edible oil industry (Section 2), which substantially reduce the protein content of the oil (Section 1).

Based on the limited data available, the Panel considers that allergic reactions to the NFI cannot be ruled out, but that the likelihood is low.

DISCUSSION

The oil obtained from *Buglossoides arvensis* (L.) is characterised by the presence of n-3 and n-6 PUFAs. The high content of SDA, an intermediate in the synthesis of EPA, is a particular feature. With the exceptions of SDA and GLA, the main fatty acids present in the NFI are widely present in common foods. The applicant intends to use the NFI in a range of food products and in food supplements to provide approximately 200 mg of SDA per day.

Upon digestion, the component fatty acids are released from the glycerides and are used primarily as an energy source. Fatty acids can also be metabolised to longer chain or more unsaturated fatty acids. ALA and SDA can be elongated and desaturated to produce EPA. In human studies in which SDA was administered in the form of SDA ethyl esters or SDA-rich oils at doses of between 750 and 4 200 mg/day for 3–16 weeks, no increase or only small increases in SDA were observed in the membranes of blood cells or in total plasma.

The compositional data provided demonstrate that the oil obtained from *Buglossoides arvensis* is similar to the oil obtained from the taxonomically related *Echium plantagineum*; refined *Echium* oil has already been authorised as an NFI. The specifications and uses of refined *Buglossoides* oil proposed by the applicant are similar to those specified for refined *Echium* oil.

The compositional data provided for three batches of the NFI, the information on the production process and the proposed specifications are considered sufficient by the Panel and do not raise safety concerns.

¹² <http://www.allergome.org>, consulted on 17 January 2013

The refining procedure applied results in a reduction of the content of PAs, a class of undesirable substances present in *Buglossoides arvensis*, to levels in the NFI below the maximum limit of 4 µg/kg as set in the specification, which corresponds to a MOE of 34 000 for children who have the highest exposure on a mg per kg body weight basis.

Erucic acid is found in the NFI at levels of around 0.2 %, which is below the maximum level of 5 % in oils and fats intended for human consumption set by EU legislation.

The refining procedure reduces the level of residual protein below the proposed limit of 10 µg/g set in the specifications.

The Panel notes that the available information does not raise concerns as regards other undesirable substances in the NFI.

Three animal feeding studies were carried out with the NFI. A number of animal studies were conducted using SDA- and/or GLA-containing oils from other sources or diets supplemented with SDA ethyl esters. No adverse effects were seen in any of these studies. The Panel notes that most studies were not designed for toxicity testing. The Panel also notes that a number of the animal studies provided were carried out in rodents. The Panel notes that the ability to desaturate and elongate precursors of PUFAs is more pronounced in rodents than in humans and therefore extrapolation of results from studies in rodents to humans requires caution. Overall, the Panel considers that available animal studies provide only limited information on the safety of the NFI.

The applicant provided human studies that investigated different plant oils or fatty acid ethyl esters as sources of SDA, GLA and ALA, which are component fatty acids of the NFI. No adverse effects were reported with up to 4 200 mg SDA/day for 12 weeks, up to 1 700 mg GLA/day for 28 days and 9 100 mg ALA/day for four weeks.

CONCLUSIONS

The Panel concludes that the novel food ingredient, refined *Buglossoides* oil, is safe under the proposed uses and use levels.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier “Application for authorisation of refined *Buglossoides* oil as a novel food” received on 16 June 2014. Submitted by Technology Crops Technology on 25 June 2013. Additional data were provided on 6 October 2014.
2. Letter from the European Commission to the European Food Safety Authority with the request for a scientific opinion on refined oil from the seeds of *Buglossoides arvensis* as a novel food ingredient. SANCO/E6/AH/ks ref. Ares(2014)1898824, dated 11 June 2014.
3. Initial assessment report carried out by the United Kingdom: “Opinion on an application under the novel foods regulation for refined oil from *Buglossoides arvensis*”, Advisory Committee on Novel Foods and Processes, Food Standards Agency.
4. Member States’ comments and objections.
5. Response by the applicant to the initial assessment report and the Member States’ comments and objections.

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ABBREVIATIONS

AI	adequate intake
ALA	alpha-linolenic acid
EPA	eicosapentaenoic acid
ESCO	EFSA scientific cooperation
FA	fatty acid
GLA	gamma-linolenic acid
LA	linoleic acid
LOD	limit of detection
MOE	margin of exposure
NDNS	national diet and nutrition survey
NFI	novel food ingredient
PA	pyrrolizidine alkaloid
PCB	polychlorinated biphenyl
PUFA	polyunsaturated fatty acid
SDA	stearidonic acid
TFA	total fatty acid