APPLICATION FOR AUTHORISATION OF REFINED BUGLOSSOIDES OIL AS A NOVEL FOOD

FULL PROCEDURE

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Summary by the applicant

Buglossoides oil is a refined vegetable oil extracted from the seeds of Buglossoides arvensis (L.) I.M.Johnst., which has not been subject to genetic modification. It is a rich source of omega-3 and omega-6 fatty acids, including the omega-3 fatty acid stearidonic acid (SDA) which is an intermediate in the synthesis of eicosapentaenoic acid (EPA) in the body from dietary alpha-linolenic acid (ALA). SDA is more efficiently converted to EPA than ALA and so dietary sources of SDA are important for individuals who are unwilling or unable to consume EPA directly (for instance from eating oily fish or fish oil supplements). There are other possible significant sources of SDA, but these are either more expensive and less concentrated (in the case of Echium oil) or are derived from a genetically modified organism and are unavailable in the EU (SDA soybean oil). Buglossoides oil therefore has the potential to improve the nutritional status of a significant subsection of the population at a lower cost than currently available products.

Buglossoides arvensis and its components have not been recorded as being used for human consumption. Buglossoides oil therefore falls under category (e) of Article 1(2) of Regulation (EC) No 258/97 (Anonymous 1997a) namely "foods or food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating and breeding practices and which have a history of safe food use." On the classification scheme established by Commission recommendation 97/618/EC (Anonymous 1997b), Buglossoides oil falls within Class 2 (Complex novel food from non-GM sources) and subcategory 2 (the source of the novel food has no history of food use in the Community).

Buglossoides oil is closely related, taxonomically, and is similar in composition, to Echium oil which is approved for sale as a food in the EU. The fatty acid profiles of the two oils are similar, with the same fatty acids being present; Buglossoides oil having a higher concentration of SDA and ALA and a lower concentration of GLA. The non-saponifiable fractions of the two oils are also similar in composition, comprising largely of sterols and tocopherols.

As a vegetable oil, Buglossoides oil comprises primarily of triglycerides (approximately 90%), with smaller quantities of diglycerides, monoglycerides, glycerol and free fatty acids. These glycerides are broken down during digestion to release the component fatty acids and so the identity of these fatty acids is important. All of the fatty acids in Buglossoides oil are present in other foods which are consumed in the EU in similar or greater proportions.

The production process is well defined and controlled, and it has been demonstrated by analysis to result in a stable and reproducible product which meets all applicable EU food requirements. The manufacturing processes are all well characterised and have been widely used in the food industry for many years. No novel processes are used.

Concern has been expressed about the potential presence of pyrrolizidine alkaloids and allergenic proteins in Echium oil. Since the source of this oil is closely related to Buglossoides arvensis, analyses were performed on representative samples of Buglossoides oil which demonstrated that both components, where present, were well below accepted levels. Other inherent constituents which might potentially give rise to toxicity (oxidation products, hydrolysis products, trans fatty acids and erucic acid,) were also each considered, analysed and found to be present at well below regulatory limits.

No significant external contaminants have been detected in the oil from analyses for pesticides, elemental contaminants, dioxin and dioxin-like polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), melamine and cyanuric acid.

No micro-organisms are used in the production of the oil. The refining of the oil involves it being held under vacuum at temperatures above 100°C for a period of time which effectively sterilises it, and the oil has a low water content and low water activity so does not support microbial life. This was confirmed by analysis of batch samples, when no microbial contamination was found.

Buglossoides oil is an inherently stable product. The primary route of degradation, as for any polyunsaturated vegetable oil, is through oxidation and, although the oxidation products are known to be harmful, the associated degradation of the taste and smell of the oil is such that it is unlikely that a seriously oxidised oil would be willingly consumed. Care is taken during manufacturing and packaging to exclude air and any of the factors which promote oxidation, and the success of this approach has been confirmed through analysis. As further confirmation, a stability study was undertaken which showed that the oil remained comfortably within specification for peroxide value for a period of eight weeks, even under severely sub-optimal storage conditions.

It is proposed that Buglossoides oil should be used in the same food groups and to an inclusion rate which will give the same level of SDA in the food as has been approved for Echium oil in the EU, with the aim of providing approximately 200mg of SDA per daily serving. This will result in a lower level of inclusion of Buglossoides oil than Echium oil because of the higher SDA content in the former. An assessment of the consumption of Echium oil has been produced, based on the indicated use levels and of food consumption data collected for the UK Food Standards Agency in the Dietary Survey Programme. These estimates represent an overestimate of the consumption of SDA from Buglossoides oil, because not all of the food groups used in compiling the estimates were represented in the final approval for Echium oil. Of the individual population groups assessed, male adults were determined to have the greatest mean and 97.5th percentile all-user intakes of stearidonic acid from echium oil on an absolute basis, at 1128 and 2175 mg SDA/person/day, respectively, while children had the lowest intakes of 719 and 1351 mg SDA/person/day, respectively (Table IX.a-2). Conversely, on a body weight basis, children were identified as having the highest intakes of any population group, with mean and 97.5th percentile all-user stearidonic acid from echium oil intakes of 51 and 103 mg SDA/kg body weight/day respectively.

These intakes are significantly lower than the intakes anticipated at the mean and 90th percentile for SDA soybean oil, from a genetically modified source which is Generally Recognised as Safe (GRAS) in the USA. This is estimated to supply up to 5200 mg SDA/day at the mean and 10500 mg SDA/day at the 90th percentile (90 and 200 mg SDA/kg body weight/day).

No groups have been identified which would be at risk through the consumption of Buglossoides oil. It is anticipated that the foods which will be displaced by Buglossoides oil in the EU are either equivalent in nutritional value but more expensive (Echium oil) or are nutritionally inferior (plant sources of ALA). Given this, it is anticipated that the nutritional status of groups who do not eat oily fish or fish oil supplements will be improved.

The metabolic fate of the oil is well understood and does not give any cause for concern.. The component fatty acids are released from the glycerides upon digestion and are used primarily as an energy source. The essential fatty acids can also be metabolised to longer chain or more unsaturated fatty acids. ALA and SDA can be elongated and desaturated to EPA, the omega-3 fatty acid typically found in fish oils. SDA has not been found to accumulate in human or animal tissues.

Buglossoides oil has been evaluated in three unpublished animal studies and no adverse effects were reported. The principal component fatty acids of the oil have been tested extensively in both

animals and humans without any ill effects. Each of the fatty acids present in the oil is also present in foods which are approved for sale in both the United States of America and Europe. A toxicological comparison with flaxseed oil as a traditional counterpart to Buglossoides oil did not reveal any cause for concern.

On the basis of the information presented in this dossier TCI believes that Buglossoides oil is a safe and wholesome food which will improve the nutritional status of people who do not habitually eat oily fish or fish oil supplements.

1 Administrative data

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Name of Novel Food:	Refined Buglossoides Oil
Date of application:	12 June 2013

2 General description

2.1 Botanical identity

Buglossoides oil is a refined edible oil obtained from the seeds of *Buglossoides arvensis* (L.) I.M.Johnst. (previously *Lithospermum arvense* L.), (NRCS 2012) an herbaceous plant which has not been genetically modified (GM). Common names include Corn Gromwell and Bastard Alkanet (Clapham *et al*. 1962). The botanical identity of the seed used for extraction of the representative oil samples listed in Appendix 1 has been confirmed by a seed testing laboratory (Appendix 2).

Buglossoides is a member of the Boraginaceae family. As a result of the upheaval which has occurred in taxonomy following the discovery of molecular methods for assessing the relationships between clades, the position of this family within the overall taxonomic scheme is in a state of flux. However, a traditional view would place the family as shown below.

Plantae – Plants
└*Tracheobionta* – Vascular plants
└*Spermatophyta* – Seed plants
└*Magnoliophyta* – Flowering plants
└*Magnoliopsida* – Dicotyledons
└*Asteridae*
└*Lamiales*
└*Boraginaceae* – Borage family

(NRCS 2012)

There is no history of use of Buglossoides oil as a food within the European Community.

On the basis of the above description, it may be concluded that Buglossoides oil falls within category (e) according to article 1 (2) of Regulation 258/97 (Anonymous 1997a) namely '*Foods or food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating and breeding practices and which have a history of safe food use.*'

On the classification scheme established by Commission recommendation 97/618/EC, Part 1, section 4 (Anonymous 1997b), Buglossoides oil falls within Class 2 ('*Complex novel food from non-GM sources*') and subcategory 2 ('*The source of the novel food has no history of food use in the Community*').

Reference will be made in this dossier to similarities between Buglossoides oil and the refined oil extracted from the seed of *Echium plantagineum* L. (Echium oil) which was approved in the European Union (EU) as a novel food in 2008 (Anonymous 2008a). Lapinskas (2012) has reported that *Buglossoides arvensis* and *Echium plantagineum* are both members of the Boraginoideae sub-family within the Boraginaceae family and are thus closely related (See also IBIS 2012, Kelley et al. 2012, NRCS 2012, Valdés 2004).

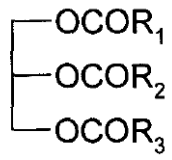
The proposed name for labelling purposes on final foods as presented to the consumer is "Refined Buglossoides oil".

2.2 Chemical identity

Buglossoides oil is a natural plant oil containing a mixture of constituents. There is therefore no specific chemical name and it has no Chemical Abstracts Services (CAS) number.

Buglossoides oil consists primarily of triglycerides with small amounts of di- and mono-glycerides, free fatty acids, and an unsaponifiable fraction, comprised mostly of sterols and tocopherols. The triglycerides are of the form shown in Figure 1 where R_1, R_2 and R_3 may be the same or different and represent various fatty acid moieties.

Figure 1 – Triglyceride structure



The fatty acids normally present in Buglossoides oil at levels greater than 1% (by fatty acid methyl ester gas chromatography (FAME GC) peak area) are shown in Table 1. The percentages of fatty acids found in representative batches are shown in Table 16 and the fatty acids listed represent over 98% of those present in the oil.

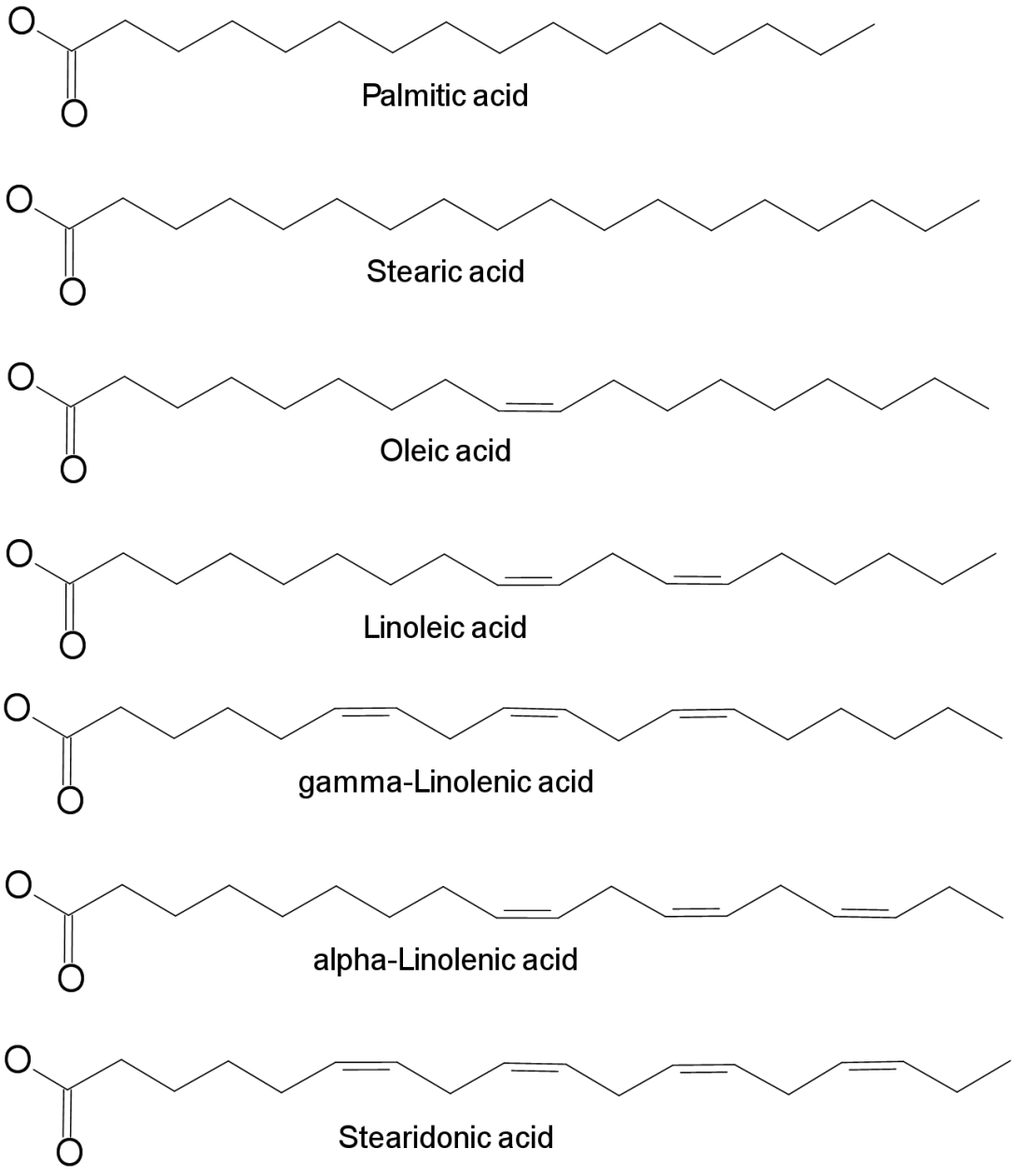
Table 1 – Identity of principal fatty acids

Common Name	Empirical formula	Scientific Name	CAS Number	Molecular weight
Palmitic acid	$C_{16}H_{32}O_2$	Hexadecanoic acid	57-10-3	256.42
Stearic acid	$C_{18}H_{36}O_2$	Octadecanoic acid	57-11-4	284.48
Oleic acid	$C_{18}H_{34}O_2$	9c-Octadecenoic acid	112-80-1	282.46
Linoleic acid	$C_{18}H_{32}O_2$	9c,12c - Octadecadienoic acid	60-33-3	280.45
gamma-Linolenic acid (GLA)	$C_{18}H_{30}O_2$	6c,9c,12c - Octadecatrienoic acid	506-26-3	278.43
alpha-Linolenic acid (ALA)	$C_{18}H_{30}O_2$	9c,12c,15c- Octadecatrienoic acid	463-40-1	278.433
Stearidonic acid (SDA), Moroctic acid	$C_{18}H_{28}O_2$	6c,9c,12c,15c- Octadecatetraenoic acid	20290-75-9	276.417

2.3 Chemical Structure

The structure of the principal fatty acids present in Buglossoides oil is shown in Figure 2.

Figure 2 – Fatty acid structures



3 Identification of essential information requirements

Buglossoides oil falls within classification 2.2 as defined in Commission Recommendation 97/618/EC (Anonymous 1997b) and, as determined from Table II therein, the following structured schemes will be followed:

I Specification of the novel food

II Effect of the production process applied to the novel food

III History of the organism used as the source of the novel food

IX Anticipated intake/extent of use of the novel food

XI Nutritional information on the novel food

XII Microbiological information on the novel food

XIII Toxicological information on the novel food

The decision trees associated with these schemes are considered in the next section.

4 Consultation of structured schemes (decision trees)

4.I Specification of the novel food

The proposed regulatory specification is given in Table 2 on page 18 below.

Buglossoides oil is a vegetable oil and as such is composed primarily of triglycerides (about 90%) with smaller proportions of di- and mono-glycerides and free fatty acids (2 – 6%, 2 – 4% and <0.3% respectively). Since these glycerides are cleaved to release free fatty acids during digestion and absorption, the fatty acid composition of the oil is normally considered to be more important, and this is indicated in Table 6. The remaining part of the oil consists of the non-saponifiable fraction (<2%) which contains a range of sterols and tocopherols. All components of the oil have been analysed in detail as described below and in Appendix 1.

The analytical data have been derived from three representative non-consecutive batches of oil produced by Technology Crops International (TCI) on their own premises in November 2012 (batch numbers NZ00053, NZ00056 and NZ00058) using the processes described in section 4.II. Batches NZ00053 and NZ00056 were extracted using the cold press process and Batch NZ00058 using hexane as a solvent. The latter was tested for the presence of residual hexane as shown in Table 15 in Appendix 1.

Refined Echium oil is approved for sale in the EU and is very similar in its composition to Buglossoides oil and so, for comparison purposes, a purchased sample of Echium oil was analysed under the same conditions. The Echium oil was extracted from consumer packs of Echiomega™ soft gelatine capsules marketed by Igennus Ltd., St. John's Innovation Centre, Cowley Road, Cambridge, CB4 0WS. The product was purchased by telephone mail order on 27 September 2012 (Appendix 2). The oil was removed from the capsules under a nitrogen atmosphere and sealed under nitrogen in a glass container prior to analysis. The commercial Echium oil analyses do not include assessment of external contaminants, as these reflect the history of the individual oil batch rather than inherent differences between the two oil sources.

The tables in Appendix 1 also show analytical data from three further batches of Echium oil taken from the regulatory dossier on which its approval as a novel food was based (Croda 2006a).

Question: *Depending on the derivation and composition of the novel food, is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?*

Applicant's answer: *Yes*

Justification

Full analytical data is provided in appendix 1.

4.I.1 Inherent constituents

A review of the toxicology of potentially toxic inherent constituents is given in section 4.III.6.

Oxidation products
Oxidation, leading to rancidity is the primary route of degradation for polyunsaturated oils and this can give rise to a number of contaminants. For this reason, several analyses have been developed and are widely used by the industry to determine the degree to which an edible oil has become oxidised. Foremost amongst these is the Peroxide Value test. The Codex standard for refined oils suggests a voluntary maximum limit of up to 10 milliequivalents of active oxygen/kg oil for refined oils (Codex 2005). As may be seen in Table 14, the Buglossoides oil batches fell comfortably below this level. TCI therefore proposes a limit of 5 meq O_2/kg for the specification of Buglossoides oil, the same as that adopted for Echium oil (Anonymous 2008a).

As a further check on the oxidative status of the oil samples, they were subjected to two additional analyses. The p-Anisidine test (Table 14) measures the long-term oxidative history of an oil. It is not specified in the Codex for vegetable oils, but the Codex Alimentarius Commission are currently considering a p-anisidine test limit of 20 for fish oils, which are particularly susceptible to oxidation (Codex 2013). The results from the Buglossoides oil samples are well below this level (Table 14). The second additional test was for epoxy fatty acids, which are oxidative degradation products; these were not found to be present in any of the samples at the limit of detection (0.1%) (Table 15).

Hydrolysis products
Another degradation route for vegetable oils is by hydrolysis, whereby a triglyceride molecule is cleaved to form a diglyceride and a free fatty acid molecule. This can be repeated to form monoglycerides, and again to form free glycerol. None of these products are toxic but their presence in an oil indicates that the quality has been compromised. The parameter which is used to assess the degree of hydrolysis is the Acid Value which measures the level of free fatty acids in the sample. The Codex suggests a voluntary limit of 0.6 mg KOH/g for refined oils and this is reflected in the same regulatory limit being specified for Echium oil (Anonymous 2008a). TCI proposes to use this limit for Buglossoides oil; the results from all three samples fall below this.

The presence of water in the oil in significant amounts promotes the hydrolysis process, but the extraction and refining processes for Buglossoides oil (involving temperatures above 90°C under vacuum) remove all but small traces of water. This has been confirmed on analysis of the three samples (Table 15) which contain less than 0.1% w/w water.

***Trans* fatty acids**
The fatty acid molecules in unsaturated fats such as vegetable oils contain double bonds, each of which can be present in one of two isomeric forms, known as *cis* and *trans*. In the principal fatty acids in common vegetable oils, the bonds are all in the *cis* form. If these oils are heated, the thermal energy can be sufficient for some of the bonds to change reversibly from the *cis* to the *trans* form, with the *trans* form being more thermally stable. Consuming a diet high in fats which contain fatty acids with *trans* bonds can raise cholesterol levels in the blood, which in turn can lead to coronary heart disease (Mensink & Katan 1990, FAO/WHO 2008). The refining process employed in the manufacture of Buglossoides oil does not use temperatures above 220°C, at which temperature the change from *cis* to *trans* bonds is negligible (De Gruyt & Kellens 2005). *Trans* fatty acids are normally associated with the hydrogenation of oils in the manufacture of margarine, where the catalyst which promotes the hydrogenation reaction also increases the rate of *cis/trans* isomerisation; this process is not used in the manufacture of Buglossoides oil. The absence of *trans* fatty acids has been confirmed by analysis of samples of Buglossoides oil (Table

15) which shows the level of fatty acids containing *trans* bonds to be below the limit of detection (1%). A limit of 2% is proposed for the regulatory specification, which is the same as that adopted for Echium oil.

Erucic acid
The level of erucic acid in the fat component of a food product is controlled within the EU to a maximum of 5% of the total level of fatty acids (Anonymous 1976). Erucic acid is found in Buglossoides oil but at much lower levels, typically around 0.2%, and this has been confirmed by analysis of the commercial samples (Table 16).

Pyrrolizidine alkaloids
Pyrrolizidine alkaloids (PAs) have been found to occur in a number of species in the Boraginaceae, including *Buglossoides arvensis* (Roeder 1999, EFSA 2011). PAs are water-soluble and therefore the bulk of any PAs present in *Buglossoides arvensis* seed would be expected to be left in the seed meal on extraction, and the level in the oil to be further reduced during refining. A sample of unrefined Buglossoides oil has been analyzed and found to contain 44 µg/kg of PAs, but refining reduces this to below 1 µg/kg, as indicated in Table 15. A maximum level of 4 µg/kg is considered to be acceptable in the EU in Echium oil (Anonymous 2008a).

Allergens
No reports of allergic reactions to *Buglossoides arvensis* have been discovered in the literature (see section 4.III). Notwithstanding this, it is prudent to ensure that protein levels are reduced as far as practicable, and this is done during processing. Protein is polar in nature and will naturally partition into an aqueous phase from a non-polar phase, such as oil. This will happen during extraction (especially when a solvent is used) and during refining, when the oil is washed with water and aqueous solutions. The oil is also filtered on at least one occasion, normally down to 1 micron, which will remove any pollen or particulate plant material. *Buglossoides arvensis* pollen is reported as being 8.8 – 11 µm across on its smallest dimension. (Perveen et al. 1995). Treatment with absorbent clay or bleaching earth would also act to reduce protein levels.

Concerns were expressed by the ACNFP committee, when they were considering the application for the approval of Refined Echium Oil as a novel food, about the allergenic potential of residual protein in the oil. In their statement of opinion, they accepted the applicant's proposal for a maximum limit of 20 µg protein/ml oil which was adopted by the European Commission in its final approval (ACNFP 2007).

The Buglossoides oil samples were initially analysed for protein content using two methods (Bradford method and chemiluminescence method) as shown in Table 15.

These results, although supportive, are not of themselves sufficient to demonstrate conclusively that Buglossoides oil meets the requirements of the Echium oil regulatory specification. The AFNCP has expressed concerns about the Bradford method in their guidance in these terms: ". . . *the Bradford Assay utilises the dye reagent Coomassie Blue to bind to protein and reacts with larger peptides and intact proteins. This means the assay performance can be affected by treatments that affect protein integrity. As a consequence, the Bradford method is not itself sufficient to confirm the absence of protein from novel foods*." (ACNFP 2011). The chemiluminescence method avoids these problems but the sensitivity of the machine used was only sufficient to demonstrate an absence of protein down to a level of 10 ppm total N, equivalent to 65 ppm of protein, which is above the limit specified for Echium oil.

Rigby et al. (2011) compared the efficiency of four different extraction methods (low-temperature acetone, phosphate-buffered saline, Olszewski bicarbonate, and sodium tetraborate) and three quantification methods (bicinchoninic acid (BCA), Bradford dye-binding, and 3-(4-carboxybenzoyl) quinolone-2-carboxaldehyde (CBQCA) fluorescence assays). They found that The bicarbonate and borate extraction methods gave the best extraction profiles, with the borate method being preferred as it was simpler, used a smaller volume of oil and allowed direct analysis of protein by the CBQCA method. The researchers then compared the variance of the results from the three analytical methods and were able to rank them with the CBQCA method giving the lowest variance (greatest precision) and the Bradford method the highest, with the differences between the methods being statistically significant at the 0.05 level. They ascribed the poorer performance of the Bradford method principally to the fact that some of the samples measured were below the lower limit of quantification for the method, but noted that the results could also be confounded because the method relies on the presence of binding sites on largely intact proteins, shows variability in responsiveness between proteins and is more susceptible to interference from other substances. The authors expressed a preference for the CBQCA method over the BCA method on the basis of its greater precision, which reflected its greater sensitivity (down to 0.075µg/ml).

Further analyses were therefore performed on Buglossoides oil samples using the borate extraction method and CBQCA (ATTO-TAG™) analytical procedure using a commercial analytical kit (Anonymous 2001) as described by Rigby et al. (2011). The results are given in Table 15 and show that the protein content of the Buglossoides oil is substantially below the limit set for Echium oil and slightly lower than that found in a commercial sample of Echium oil.

TCI believes that the absence of reports of allergenic activity in *Buglossoides arvensis* together with the fact that Buglossoides oil, when prepared using the methods described, does not contain sufficient protein to pose a significant allergenic risk even if it were to contain allergens. They therefore propose that the limit for protein in Buglossoides oil should be set at 20 µg protein/ml oil, the same limit as that adopted for Echium oil.

Unsaponifiable matter
The major components of the unsaponifiable fraction are sterols and tocopherols (Table 15), neither of which are generally considered to be toxic These have been characterised as shown in

Table 17 (phytosterols) and Table 18 (tocopherols). The principal sterols present in both oils are campesterol and β-sitosterol, with smaller proportions of other sterols. Of these, all were either found at a greater concentration in commercial Echium oil than in the Buglossoides oil or are found in other commonly consumed foods. The US Food and Drug Administration (FDA) has determined that consumption of phytosterols may reduce the risk of heart disease (FDA 2005) although no such health claim will be made for Buglossoides oil on the basis of its sterol content.

Tocopherols and tocotrienols are components of vitamin E and are essential for good health. Only γ-tocopherol was found in Buglossoides oil at significant levels (Table 18).

4.I.2 External contaminants

Pesticides
It is possible that approved agrochemical products may be applied to *Buglossoides arvensis* as coatings on sowing seed, while the crop is growing or post-harvest, in which case these will be

applied in accordance with Good Agricultural Practice and with the label recommendations, and in compliance with local regulations.

A pesticide screen was carried out on the three samples of Buglossoides oil and no residues were found at or above the limit of detection (Table 19).

Elemental contaminants
No significant residues of elemental contaminants were found in the Buglossoides oil samples, as shown in Table 19, and in all cases levels were substantially below those required in EU legislation.

Dioxin and dioxin-like polychlorinated biphenyls (PCBs)
The results of the analyses on Buglossoides oil are given in Table 20 which shows that in all three samples the levels of dioxins and PCBs are below the level required by EU regulations (Anonymous 2006).

Polycyclic Aromatic Hydrocarbons (PAHs)
Only one PAH (phenanthrene) was detected in the three samples of Buglossoides oil and the concentration was close to the limit of detection (Table 21). No PAHs of concern were detected and the levels of detection were at or below the limits set under EU regulations (Anonymous 2006).

Melamine and cyanuric acid
Melamine and cyanuric acid are associated with fraudulent adulteration of products and have been primarily seen in milk and pet food. Neither compound was found in any sample of Buglossoides oil, with a level of detection substantially below the regulatory limit (Table 19).

4.I.3 Nutrients

Buglossoides oil is composed primarily of triglycerides with small amounts of di- and mono-glycerides, free fatty acids, glycerol and a non-saponifiable fraction; the results of analyses on three samples of oil are given in Table 15. During digestion, the glycerides are broken down to release the fatty acids so, from a nutritional perspective, the fatty acid composition is also important. This is given in Table 16. As noted in section 4.I.1, the sterol fraction, tocopherols and tocotrienols may also be considered to be nutrients. The analysis results for these components are given in Table 17 and Table 18 respectively. These results indicate that Buglossoides oil and Echium oil are broadly similar, except for the increased levels of ALA and SDA and reduced level of GLA in Buglossoides oil.

Question: *Is the information representative of the novel food when produced on a commercial scale?*

Applicant's answer: Yes

Justification

The analytical data presented under the previous question was derived from three non-consecutive batches of Buglossoides oil produced at the kilogram scale in TCI's laboratory using equipment which closely adheres to the timing and conditions used at the commercial scale. The extraction

and refining techniques employed are standard in the food oil industry and are well characterised and understood. The oil batches can therefore be expected to be representative of product produced at commercial scale.

Question: *Is there an appropriate specification (including species, taxon etc. for living organisms) to ensure that the novel food marketed is the same as that evaluated?*

Applicant's answer: Yes

Justification

The proposed regulatory specification for Buglossoides oil is given in Table 2. This specification is the same as that which has been approved for Echium oil except that the level of stearidonic acid has been increased from 10% to 15%, which reflects the higher concentration normally found in this species.

Table 2 also shows the results from analyses reported more fully in appendix 1, which demonstrate that three representative non-sequential batches which were manufactured during November 2012 meet the proposed specification.

Table 2 – Proposed regulatory specification

Parameter	Proposed specification	Buglossoides oil batches		
		NZ00053 Batch 4	NZ00056 Batch 5	NZ00058 Batch 6
Description	Buglossoides oil is the pale yellow product obtained by refining oil extracted from the seeds of *Buglossoides arvensis* (L.) I.M.Johnst.	Confirmed	Confirmed	Confirmed
Stearidonic acid content	Not less than 15% w/w of total fatty acids	20.5	19.7	20.8
Trans fatty acids	Not more than 2% w/w of total fatty acids	<1.0	<1.0	<1.0
Acid value	Not more than 0.6 mg KOH/g	0.22	0.12	0.34
Peroxide value	Not more than 5 meq O_2/kg	2.03	1.55	1.22
Unsaponifiable content	Not more than 2%	0.28	0.43	0.73
Protein content (total nitrogen)	Not more than 20 µg/ml	1.3	1.0	1.3
Pyrrolizidine alkaloids	Not detectable with a detection limit of 4 µg/kg	<1	<1	<1

4.II Effect of the production process applied to the novel food

Question: Does the novel food undergo a production process?

Applicant's answer: Yes

Justification

Buglossoides oil is manufactured from seed in accordance with cGMP using food-grade raw materials and processing agents. Traditional food oil manufacturing processes are used which do not alter the fatty acid profile of the oil. Schematic diagrams of the manufacturing process are provided in Figure 3 and Figure 4 below. These processes are very similar to those used for the production of Echium oil (Croda 2006a, NFU 2009, NFU 2010).

4.II.1 Seed production

Buglossoides arvensis is a wild plant which has not hitherto been domesticated. It is native in England and is found in many parts of Europe and North America (Clapham et al. 1962, NRCS 2012). It has been classified as "can be weedy or invasive" in the USA but is not considered to be noxious (NRCS 2012). It can be controlled by a range of herbicides (Haskins et al. 2012) and so it is not anticipated that it will become a troublesome weed in areas where it is grown as a crop. Since it is already extant in these areas, its production is unlikely to cause any ecological disturbance.

The seed is grown by farmers in conventional field crops using standard agricultural techniques and using standard agricultural equipment, following Good Agricultural Practice, or the local equivalent. Pesticides may be applied to the crop according to need, but only those which are permitted locally and then only in accordance with the manufacturer's instructions. The farmers are contracted to TCI or to a production company acting on its behalf and crops are inspected during the growing season. The resultant seed is cleaned, dried and transported to the extraction facility.

4.II.2 Extraction of Buglossoides oil

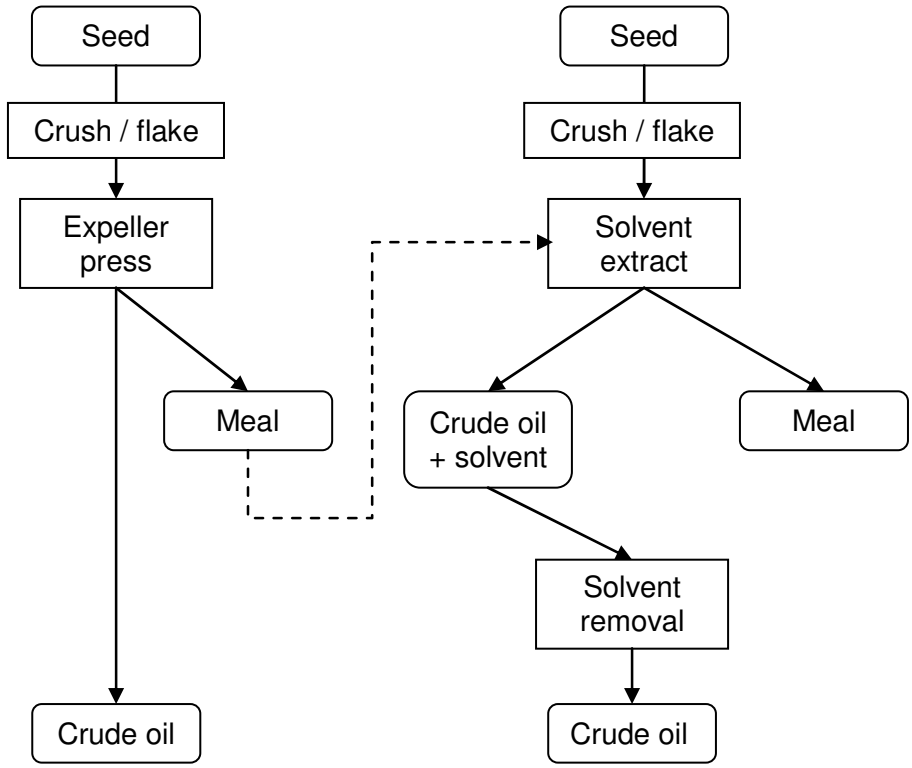
Oil is normally extracted from the seed of *Buglossoides arvensis* by one of two methods, both of which are conventional industry standard.

Option A: Expeller pressing. The seed is first prepared by crushing or flaking and then passed to a screw press expeller which subjects the seed material to high pressures to expel the oil. The temperature of the oil during extraction is normally in the range 50 - 85°C, depending on the design of the press and the rate of operation. The oil is collected, filtered and transferred to storage containers under nitrogen or other inert gas.

Option B: Solvent extraction. The seed is first 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.

The meal remaining after expeller pressing contains residual oil, so the meal is commonly re-extracted using option B.

Figure 3 – Extraction process for Buglossoides oil



4.II.2 Refining of Buglossoides oil

After extraction, crude Buglossoides oil may be refined by several methods as indicated in Figure 4 and described below. This represents a relatively mild form of refining and all of the processes described are standard in the edible oil industry and have been used without any safety concerns for many years (See, for instance, Anderson 2005).

Some treatments may be repeated one or more times if necessary to bring an individual batch of oil into specification.

The methods used for extraction and refining will remove unwanted material from the oil but will not significantly alter its composition.

Degumming (acid treatment, water treatment and centrifugation)
In the first step, phospholipids are removed by the 'degumming' process which involves mixing the oil with water or an acid solution to form gums, which are removed by centrifugation.

Addition of sodium hydroxide
A dilute sodium hydroxide solution may be added after degumming in order to neutralise free fatty acids in the oil and previously added citric or phosphoric acid. The quantity of sodium hydroxide is calculated to allow for complete neutralisation of any acid already added to the oil as well as the free fatty acids and will thus vary from batch to batch. In cases where the level of free fatty acids is already within specification, the calculated amount of sodium hydroxide required may be zero, in which case the step is omitted. The free fatty acids are converted to sodium soaps which are then

separated by centrifugation. As the quantity of sodium hydroxide is calculated according to the acid content of each batch, and as it is hydrophilic and will be preferentially taken up in the aqueous phase during processing, it follows that little if any of the chemical will remain in the oil after centrifugation, and any residues are further reduced by washing the oil with water and during the bleaching operation which follows. Sodium hydroxide neutralisation is commonly used during the refining of food-grade vegetable oils.

Bleaching and filtration
Removal of colour, oxidising matter, trace amounts of residual gums, and metallic salts from oil is performed with bleaching earths which adsorb the impurities. The adsorbents holding the impurities are then separated from the oil by filtration. Bleaching is performed at a temperature in excess of 90°C under vacuum and with agitation. Moisture present in the oil vaporises in the vacuum and the process substantially eliminates any microbiological contamination in the oil. After bleaching, the bleaching earth is filtered out of the oil.

Packaging & storage
The finished oil is transferred to the desired containers, which are filled to the desired weight and the headspace is flushed with nitrogen prior to being sealed. Containers are then labelled and placed in a controlled storage area until shipped to the customer.

Optional processes
Optionally, on some batches, the following processes may also be carried out. The finished product will still meet the specification provided in Table 2.

Addition of citric acid during bleaching
Citric acid may be added to the oil with the bleaching clay to act as a chelating agent to help remove metals and improve the removal of any residual soaps that may be present if sodium hydroxide had been used after degumming. The citric acid may also help to protect oil quality during the bleaching process because of its antioxidant properties.

Addition of cellulosic filter aids during filtration
Filtration of the oil may be improved when cellulosic filter aids are added to the oil. The filter aids form a “filtering cake” on the screens or membrane of the filter equipment and assist in removal of impurities and prevent the filter from becoming “blinded” or plugged.

Addition of activated carbon during bleaching
If oil contains high levels of chlorophyll, metals or other contaminants, activated carbon may be added to the oil (normally at the same time as the bleaching earth) to assist in their efficient removal. The activated carbon is removed during the filtration stage after bleaching

Addition of silica gel during bleaching
The addition of silica gel to the oil may assist in the removal of impurities such as soaps, phospholipids, and trace metals. The silica has a high adsorption capacity and traps polar impurities from the oil. The spent gel is removed during the filtration stage after bleaching.

Deodorisation after bleaching
Deodorisation is generally part of the manufacturing process but in many batches the previous steps in the oil refining process can be effective in removing all contaminants prior to deodorization. In the event a batch of oil fully meets specifications after bleaching, then deodorization may not be required, and the oil would proceed to the finished oil tank as is. However, when required to meet specifications, deodorization can be used to remove odour and flavour compounds, some coloured matter, as well as sterols and free fatty acids. The deodorization process is carried out under high

vacuum and temperatures in excess of 130°C but less than 220°C with live steam injection through the oil. When deodorisation is complete, the deodorised oil is cooled and the vacuum broken with nitrogen.

Winterisation
If waxes are present at high levels in the oil, removal of waxes from oil is performed by chilling the oil, which cases the waxes to crystallise so that they can be removed by filtration. Depending on waxes present, Perlite may be added to the oil to enhance crystal growth and improve filterability.

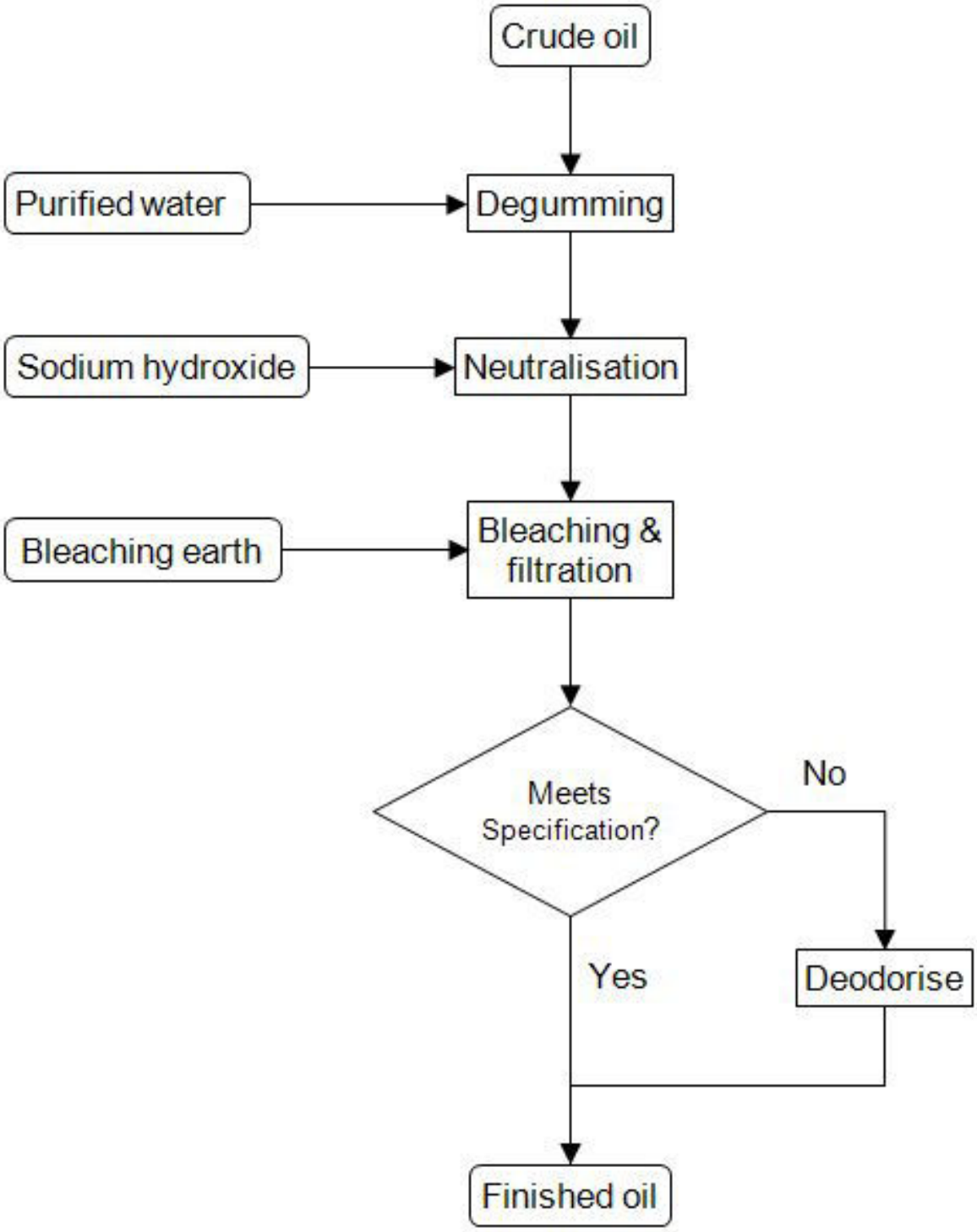
Addition of antioxidants in finished oil
To improve the stability of the finished oil, antioxidants may be added that are approved food additives in the EU, such as those shown in Table 3. The antioxidant protects and improves the stability of the oil by inhibiting oxidation. The quantity of antioxidant added will be at or below the applicable regulatory maximum limit.

All processing aids and additives employed in the manufacture of Buglossoides oil are listed in Table 3. They meet food-grade specifications and are used in compliance with appropriate European regulations and current good manufacturing practice (cGMP).

Table 3 – Processing aids and additives

Processing Aids	Details
Hexane/Isohexane	Extraction solvent
Citric Acid	Chelating agent and antioxidant
Sodium Hydroxide	Neutralising agent
Phosphoric Acid	Agent for removal of phosphatides
Bleaching Clay	Adsorbent
Perlite	Filter aid
Cellulosic filter aids	Filter aids
Activated carbon	Adsorbent
Silica gel	Adsorbent
Antioxidants and synergists	Approved antioxidants may include *tert*-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate, ascorbyl palmitate, ascorbic acid, tocopherols, rosemary extract, propylene glycol and citric acid

Figure 4 – Refining process for Buglossoides oil



4.II.3 Monitoring

The seed used for producing Buglossoides oil is produced under contract to TCI or to seed production companies acting on its behalf. The terms of the contract allow for all crops to be visited by the TCI's representatives at least once during the growing season. All harvested seed is tested for purity and moisture and crop identity prior to delivery. Buglossoides oil is manufactured in the TCI's own factory or by manufacturers under contract to him, and he maintains ownership of the material throughout processing. Manufacture is in conformance with cGMP and with ISO 22000:2005 (which incorporates the Hazard Analysis and Critical Control Points (HACCP) system). These processes ensure that the product is monitored throughout the production process. Once processing is complete the oil is tested against the product specification by either the in-house Quality Control laboratory or by external analysts working to similar standards and under the control of the TCI. In addition, a sampling system for random batches of oil will be employed to monitor a much wider range of parameters, including the levels of undesirable substances such as PCBs, dioxins and pesticides. Records are kept in order to ensure full traceability of each finished batch of oil back to the individual farms on which the seed was grown.

In the event that a batch of oil is found to be out of specification after processing, it is not released by Quality Control and the following procedure for the reprocessing of material is followed:

- If the material failure is considered to be remediable, then the batch will be reprocessed using some or all of the methods described above or blended with another batch of material and then reprocessed in order to generate a product that will again be tested against the specification.
- If the failure is not considered to be remediable, the material will be disposed of in accordance with the appropriate regulations.

4.II.4 Stability

As noted in section 4.I.1 above, the primary degradation route for Buglossoides oil is by oxidation. Polyunsaturated oils such as Buglossoides oil are more susceptible to oxidation than monounsaturated or saturated oils and fats, and so more care is required to protect them. Immediately after refining, Buglossoides oil is packed in airtight containers and so the degree to which the oil may oxidise is largely determined by the amount of oxygen which is included with the oil at the time of packing, either as dissolved air or as residual air in the headspace. The bleaching process involves holding the oil for a period under vacuum which substantially reduces the level of dissolved air during refining, and the containers are normally filled to capacity (to reduce the headspace) and the headspace is flushed with nitrogen gas, both of which will reduce the degree of exposure to air.

The rate of oxidation is determined by a number factors, principally the temperature and the level of efficacy of any antioxidants which may be present. Buglossoides oil is normally stored at cool ambient temperature. There are some natural antioxidants present in the oil (primarily γ-tocopherol, as shown in Table 18) and additional antioxidants may be added to further enhance the stability for particular applications.

The rate of oxidation can be increased by exposure to light and by the presence of certain metallic contaminants, particularly iron and copper. Buglossoides oil is stored in light-proof containers and care is taken during manufacture to avoid contamination with metals. As a result, the level of metallic contamination in the product is very low, as is demonstrated in Table 19. Oxidation rate can also be increased by the presence of certain enzymes, but these are removed from Buglossoides oil during the refining process as evidenced by the extremely low levels of residual protein shown in Table 15.

Oxidation in vegetable oils such as Buglossoides oil is not a significant safety hazard, even though the compounds produced have some toxicity associated with them. This is because, by the time that the oxidation has reached a stage where potentially toxic compounds are produced, substantial amounts of volatile compounds have also been released which make the oil smell and taste disgusting – the oil is rancid. Therefore, any product containing oil which has reached this level of oxidation would be rejected by a potential consumer on organoleptic grounds.

To confirm the stability of Buglossoides oil, a study was undertaken in which samples of oil from three production batches were placed in sealed air-tight glass vials with nitrogen gas in the headspace. Half of the vials also received 1000 ppm of natural mixed tocopherols containing a minimum of 700 mg/g of d-γ-tocopherol as an antioxidant, in compliance with EU additives legislation (E306), giving a total of six vials for each sampling point. The vials were then stored at either 4℃, 22℃ or 60℃ in the dark. Vials were periodically removed from storage, allowed to reach ambient temperature and the oil analysed for peroxide value. The results to date are shown in Table 4.

Table 4 – Oxidation levels in Buglossoides oil during storage

Test	Temp	Anti-oxidant	Buglossoides oil samples					
			NZ00070		NZ00073		NZ00074	
			Start	8 weeks	Start	8 weeks	Start	8 weeks
Peroxide value	4℃	-	0.36	0.75	0.41	0.88	0.37	1.01
		+	0.36	0.81	0.41	0.78	0.37	0.93
	22℃	-	0.34	1.41	0.31	1.20	0.41	1.36
		+	0.34	1.14	0.31	0.95	0.41	1.31
	60℃	-	0.26	0.78	0.30	0.59	0.34	0.54
		+	0.26	0.59	0.30	0.90	0.34	0.62

\+ Includes 1000 ppm mixed tocopherols
\- No tocopherols

The results show that the oil remains within specification for peroxide value for a period of 8 weeks at all temperatures. This confirms that the oil is stable, even when stored under extreme conditions.

The storage of the oil samples in sealed vials purged with nitrogen closely mimics, at a smaller scale, the storage conditions for bulk oil after manufacture. It is also a good model for oil packed in softgel capsules (which form an excellent barrier against air) and for oil supplied to the consumer in sealed bottles (for instance, as a salad oil variant of a dietary supplement). For

other uses as a food ingredient, 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 data show that there is little deterioration of the oil in sealed vials after 8 weeks even when stored at seriously sub-optimal conditions. Since the principal route for degradation is oxidation, this will mostly take place at the beginning of the storage period during which time the small amounts of dissolved oxygen will be consumed. TCI therefore believes that this oil is sufficiently stable to be used in consumer products with appropriately calculated shelf lives.

Question: Is there a history of use of the production process for the food?

Applicant's answer: Yes

Justification

The production processes used for manufacturing Buglossoides oil have long been standard in the edible oil industry. See for instance FAO (1994) and Anderson (2005).

4.III History of the organism used as the source of the novel food

Question: Is the novel food obtained from a biological source, i.e. a plant animal or microorganism?

Applicant's answer: Yes

Justification

The product is obtained from a plant, as described in section 2.1.

Question: Has the source of the novel food been derived using GM?

Applicant's answer: No

Justification

The varieties used to produce Buglossoides oil have not been genetically modified.

Question: Is the source organism characterised?

Applicant's answer: Yes

Justification

Buglossoides arvensis was first described and classified (as *Lithospermum arvense*) by Linnaeus (1753). It has been described more recently by Clapham *et al.* (1961) as:

> An erect pubescent slightly rough annual 10-50 (-90) cm. Stems usually simple or little branched. Leaves up to 3(-5) cm., the lower obovate, obtuse, narrowed into a petiole, the upper linear-lanceolate or oblong-lanceolate, acute or subacute, sessile, lateral nerves not apparent. Cymes terminal, short. Corolla not much exceeding calyx, 3-4 mm, diameter, white, rarely bluish, tube violet or rarely blue. Nutlets trigonous-conical, greyish-brown, warty, Flowers May - July. Chromosome number 2n=38. Therophyte.
>
> Native to the UK. Mainly in arable fields. .Fairly common in England, rare and perhaps not native in the rest of the British Isles. Europe, probably not native in the north; Asia to North West India.

The taxonomy of *Buglossoides arvensis* has been described in section 2.II.

Question: Is there information to show that the source organism and/or foods obtained from it are not detrimental to health?

Applicant's answer: Yes

Justification

The safety of Buglossoides oil is supported by consideration of the safety of its constituents which are found in a wide range of other food products and which have been tested in both animals and humans, as well as by studies on the whole oil in animals.

4.III.1 Regulatory status

Buglossoides oil itself is not yet approved for sale in any country. However, the components of the oil are found in a range of common foods and are in some cases approved for use in their own right. The use of these components in the food supply without reports of adverse effects is indicative of their safety.

In Europe palmitic acid, stearic acid and oleic acid are approved for use as a food additives in their own right (as E570). All of the fatty acids present in Buglossoides oil may be used as food additives in the form of mono- and diglycerides (designated as E471) as they are all present in food oils and fats, as described below (Anonymous 2012). Both additives are permitted in a very wide range of foodstuffs following the '*quantum satis*' principle, which is to say that:

> ". . .no maximum level is specified. However, additives shall be used in accordance with good manufacturing practice, at a level not higher than is necessary to achieve the

> intended purpose and provided that they do not mislead the consumer."(Anonymous 1996)

Echium oil, which is also a triglyceride vegetable oil and which contains all the fatty acids present in Buglossoides oil in similar proportions has been approved as a food in the EU (Anonymous 2008a).

In the United States, there are no FDA regulations that specifically address the use of Buglossoides oil, SDA, GLA or ALA as ingredients in food. Certain uses of palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid and stearidonic acid (or materials containing substantial concentrations of them) as food ingredients have been affirmed or notified as generally recognised as safe (GRAS) in the USA as shown in Table 5. SDA soybean oil has been notified as GRAS without objection from the FDA and so may legally be sold for food or supplement use (OFAS 2009b). Echium oil has been notified as a new dietary ingredient without objection from the FDA and so may legally be sold as a dietary supplement in liquid or encapsulated form (Croda 2006b, Anonymous 2008b). Both oils contain the same fatty acids as those found in Buglossoides oil.

Table 5 – Components of Buglossoides oil affirmed or notified as GRAS

Component	CFR/GRN code	Use
Palmitic acid	21 CFR 184.1065	In food with no limitation other than cGMP
	21 CFR 184.1329	In food with no limitation other than cGMP (as Glyceryl palmitostearate)
	GRN 248 (Compass Foods 2008, OFAS 2008)	Sucrose fatty acid esters (i.e., sucrose monoesters of lauric acid, palmitic acid, and stearic acid) manufactured by reaction of sucrose with vinyl esters of lauric, palmitic, and stearic acids
Stearic acid	21 CFR 172.860	In food as a lubricant, binder or defoaming agent in accordance with cGMP
	21 CFR 184.1505	In food with no limitation other than cGMP (as mono- and diglycerides)
	GRN 248 (Compass Foods 2008, OFAS 2008)	Sucrose fatty acid esters (i.e., sucrose monoesters of lauric acid, palmitic acid, and stearic acid) manufactured by reaction of sucrose with vinyl esters of lauric, palmitic, and stearic acids
Oleic acid	21 CFR 172.862	In food as a lubricant, binder or defoaming agent in accordance with cGMP
	21 CFR 184.1505	In food with no limitation other than cGMP (as mono- and diglycerides)
	GRN 306 (Monsanto 2009b, OFAS 2010)	Soybean oil with reduced palmitic and linolenic acids and increased oleic acid
Linoleic acid	21 CFR 184.1065	In food with no limitation other than cGMP
	21 CFR 184.1505	In food with no limitation other than cGMP (as mono- and diglycerides)
alpha-Linolenic acid	GRN 256 (Polar Foods 2008, OFAS 2009a)	High linolenic acid flaxseed oil
gamma-Linolenic acid	GRN 283 (Monsanto 2009a, OFAS 2009b)	Stearidonic acid soybean oil
Stearidonic acid	GRN 283 (Monsanto 2009a, OFAS 2009b)	Stearidonic acid soybean oil

GRN = Generally Recognized as Safe Notification

4.III.2 Natural presence in the diet

Buglossoides oil has no history of use in food and, therefore, is not normally present in the diet. However, the component fatty acids are commonly found in a wide range of foods. (All percentage values quoted below represent the given fatty acid as a proportion of the total fatty acids present in the source oil.)

The fatty acids normally present in Buglossoides oil at levels greater than 1% (peak area) are shown in Table 6, in comparison to the principal fatty acids found in other food oils (Gunstone 2005). The data for Buglossoides oil was measured on samples taken from a range of genotypes, a range of seasons and a range of environments. These observed ranges were then widened to allow for anticipated improvements in the crop arising from research into agronomy and conventional plant breeding.

Table 6 – Fatty acid profiles

Fatty acid	Buglossoides oil	Cocoa butter	Corn oil	Flaxseed oil	SDA soybean oil[1]	Echium oil[2]
Palmitic 16:0	4.5 – 7.5	26	9 – 17		9 – 13	6.0
Stearic 18:0	1.5 – 4.0	34			2.0 – 5.5	3.5
Oleic 18:1	4.0 – 10.0	35	20 – 42	16	10 – 20	17.2
Linoleic 18:2	7.0 – 15.0		39 – 63	24	15 – 30	18.6
GLA 18:3 n-6	3.5 – 8.5				5 – 8	10.2
ALA 18:3 n-3	35 – 55			50 – 60	9 – 12	29.5
SDA 18:4 n-3	15 – 30				15 – 30	12.6

All figures are expressed as percentage of total fatty acids.
[1] Monsanto (2009a) SDA soybean oil is a genetically modified product available for sale in the USA.
[2] Mean values calculated from Croda (2006a) Table XI.a-1

Gunstone (2005) and Haas (2005) give further examples of the contents of these fatty acids in a range of common food oils as follows:

> Palmitic acid is found in beef tallow (20.9–28.9 %), chicken fat (23.2%), palm oil (44%) and soybean oil (11%)
>
> Stearic acid is found in beef tallow (7.0–26.5%), and chicken fat (6.4%),
>
> Oleic acid is found in beef tallow (30.4–48.0%), chicken fat (41.6%), olive oil (78%), rapeseed (canola) oil (56%), soybean oil (22%) and sunflower oil (20%)

Linoleic acid is found in groundnut oil (41%), rapeseed (canola) oil (26%), soybean oil (53%) and sunflower oil (60%)

Alpha-linolenic acid is found in rapeseed (canola) oil (10%) and soybean oil (10%).

Alpha-Linolenic acid (ALA) is an omega-3 essential fatty acid and is the parent compound for the omega-3 series of fatty acids. It is converted in the human body to SDA and thence to eicosapentaenoic acid (EPA) and, to a lesser extent, docosahexaenoic acid (DHA). As such it is an important component of the diet, both to prevent nutritional deficiencies and to reduce the risk of chronic cardiovascular disease (de Lorgeril & Salen 2004, Gebaur et al. 2006). The American Heart Association recommends consumption of foods containing ALA such as tofu and other forms of soybeans, canola, walnut and flaxseed, and their oils with the aim of consuming 1.3 – 2.7 g per day of ALA (Kris-Etherton *et al*. 2002, AHA 2010). Hiomega™ flaxseed oil, which is GRAS, provides up to 25g of ALA per day (Polar Foods 2008, OFAS 2009a). Most Western diets provide more than 10 times more omega-6 than omega-3 fatty acids. There is general agreement that individuals should consume more omega-3 and less omega-6 fatty acids to promote good health (ODS 2005).

Gamma-linolenic acid (GLA) is an omega-6 fatty acid and is an intermediate in human metabolism in the synthesis of dihomo-gamma-linolenic acid (DGLA) and arachidonic acid from the omega-6 parent compound, linoleic acid (LA) (Kapoor and Huang 2006). It is estimated that adult humans synthesise between 100 and 1,000 mg/day of GLA from dietary LA (Horrobin 1992). GLA (together with its metabolite DGLA) is present in human breast milk at a level of 100 to 400 mg/litre and a fully breast-fed infant may be consuming 20 to 80 mg/kg/day of GLA+DGLA. GLA is present in small quantities (less than 0.25%) in the lipids from beef, chicken and egg yolk (Horrobin 1992).

GLA is found in a number of oils and foods which are used as dietary supplements. It was estimated in 1990 that one million kilograms of evening primrose oil (*Oenothera biennis L*.) had been sold since the mid 1970s, with a GLA content of 7-10%, equivalent to approximately one million person-years of consumption. No major adverse events had been reported (Horrobin 1990). Borage oil, also known as Starflower oil, (*Borago officinalis* L.) contains 23 – 26% GLA and blackcurrant seed oil (*Ribes nigrum* L.) contains 15 – 20% (Horrobin 1990, Sarubin-Fragakis & Thomson 2007). Hempseed oil (*Cannabis sativa* L.) is reported to contain 4% GLA (Callaway 2004); the cyanobacterium *Spirulena platensis* contains 18-21% and the fungus *Mucor javanicus* contains 15 - 18% (Lindemann and Merolli 2006). Echium seed oil (*Echium plantagineum* L.) contains 10 -11% GLA and has been approved in the European Union as a novel food ingredient for use in milk-based products and drinkable yogurt products delivered in a single dose, cheese preparations, spreadable fat and dressings, breakfast cereals, food supplements, dietary foods for special medical purposes, and foods intended for use in energy-restricted diets for weight reduction (Anonymous 2008a). Echium oil has also been notified as a New Dietary ingredient (NDI) in the USA without raising any questions from the FDA (Croda 2006b, Anonymous 2008b). Sonova 400® is a modified safflower variety (*Carthamus tinctorius* L.) which contains 45% GLA in its seed oil. It was notified as a new dietary ingredient (NDI) to the FDA in 2009 and has been marketed since June 2010 (Watkins 2010).

Stearidonic acid (SDA) is an omega-3 fatty acid and is an intermediate in human metabolism in the synthesis of eicosapentaenoic acid (EPA) from the omega-3 parent compound ALA. EPA and docosahexaenoic acid (DHA) are important components in the diet. The American Heart

Association recommends eating two servings of fatty fish per week (to achieve an intake of 0.3 – 0.5 g per day of EPA plus DHA) in order to reduce the incidence of cardiovascular heart disease (CVD) in the general population. They recommend 1 g per day for patients with documented CHD and up to 4 g per day for patients needing triglyceride lowering (Kris-Etherton *et al*. 2002). Dietary SDA has been shown to be 17% to 30% as effective as dietary EPA in raising EPA levels in red blood cells and plasma phospholipids (Whelan 2009).

Stearidonic acid (SDA) is also found in a variety of foods and ingredients. Fish and other seafood is a major source, normally containing 0.5 – 2.0% of the total fatty acids, although the content can be as high as 7% (Ackman 2005). SDA is uncommon amongst terrestrial plants but is found in blackcurrant seed oil (*Ribes nigrum* L.) at 2 – 6% (Whelan 2009). Hemp seed oil also contains SDA, at levels between 0.4% and 1.9% (Callaway *et al*. 1996). The richest dietary source is echium oil which, as has been noted above, is available in the European Union as an ingredient in a range of foods and in the USA as a dietary supplement. It contains at least 10% SDA.

The Monsanto company has developed a modified soybean variety which produces a seed oil containing 9-12% ALA, 5-8% GLA and 15-30% SDA. In 2009, Monsanto provided notification to the FDA that it considered this oil to be GRAS and the FDA raised no questions (Monsanto 2009a, OFAS 2009b). The company was reported as planning to supply commercial quantities of the product (Soymega™) in 2012 and that it would be incorporated into a wide range of foodstuffs including nutrition bars, salad dressings, pasta sauces and margarine (Watson 2011).

In summary, the main fatty acids present in Buglossoides oil are already present in the food supply. The safety of other constituents of Buglossoides oil is addressed in section 4.III.6.

4.III.3 Toxicology

The toxicological safety of Buglossoides oil has been established primarily through the testing of its main constituent fatty acids, both individually and in the form of vegetable oils from other species. In support of this evidence, there are also results from three studies in two species on Buglossoides oil itself.

4.III.3.1 Absorption, distribution, metabolism and excretion

Like all dietary vegetable oils, the primary fate of the fatty acids in Buglossoides oil is beta-oxidation for use as energy. The essential fatty acids can also be metabolised to longer chain or more unsaturated fatty acids. Both the ALA and SDA in Buglossoides oil can be elongated and desaturated to EPA, the omega-3 fatty acid typically found in fish oils. Pre-clinical and clinical studies all show that dietary supplementation with either ALA or SDA results in an enrichment of tissue phospholipids with EPA, although the efficiency of this conversion is greater with SDA than with ALA (James et al. 2003).

Human and animal studies show that dietary SDA does not accumulate in tissues. The consumption by humans of 0.75g SDA ethyl esters/day for 3 weeks followed by 1.5g/day for an additional 3 weeks resulted in no measurable impact on erythrocyte, platelet or mononuclear leukocyte SDA content, nor on plasma cholesterol ester and triglyceride SDA content (James et

al. 2003). Similarly, the consumption by hypertriglyceridemic subjects of 15g echium oil per day containing 1.9g SDA and 4.3g ALA for 28 days resulted in no change in peripheral blood neutrophil SDA levels and only a slight increase in total plasma SDA to 0.2% of total plasma fatty acids (Surette et al. 2004). Similar results were obtained using SDA-enriched soybean oil where Harris et al. (2008) and Lemke et al. (2010)) measured no increase or small increases in erythrocyte SDA levels not exceeding 0.05% of total cellular fatty acids after consuming 4.2g SDA/day for 12 weeks and 3.66g SDA/day for 16 weeks, respectively.

4.III.3.2 Preclinical toxicology Studies

Three unpublished preclinical studies on Buglossoides oil, the published studies involving SDA and a selection of studies on GLA are summarised in Table 7.

Table 7 – Summary of preclinical toxicology studies

Citation	Objective	Subjects[1]	Test article	Dose	Duration	Results
Surette and Matar 2012, Surette 2013	Subchronic oral toxicity	10F BALB/c mice	Buglossoides oil	2.6% oil in diet (approx 3.9 g/kg/day)	28 days	No adverse effects reported
Surette and Matar 2012	Subchronic oral toxicity	30F BALB/c mice	Buglossoides oil	2.6% oil in diet (approx 3.9 g/kg/day)	Up to 56 days	No compound-related adverse effects reported
Plante & Surette 2012	Subchronic oral toxicity	60 M+F salmon fry	Buglossoides oil	11.5% in diet	56 days	No adverse effects reported
Hammond *et al.* 2008	Subchronic oral toxicity	30M + 30F Sprague–Dawley Crl:CD(SD) rats	20% SDA soybean oil	Up to 3g oil/kg/day by gavage	28 days	NOAEL = c.0.6 g/kg/day SDA[2] (highest level tested)
Hammond *et al.* 2008	Subchronic, reproductive & developmental oral toxicity	25M + 45F Sprague–Dawley Crl:CD(SD) rats	26% SDA soybean oil	Up to 4g oil/kg/day in diet (variable percentage)	90 days	NOAEL = c.1.0 g/kg/day SDA[3] (highest level tested)
Engler 1993	Subchronic oral toxicity	16-19 M spontaneously hypertensive rats	Blackcurrant oil	11% of oil in the diet (approx. 330 mg SDA/kg/day & 880 - 2860 mg GLA/kg/day)	49 days	No compound-related adverse effects reported
Barzanti *et al.* 1995	Subchronic oral toxicity	16M Wistar rats	Blackcurrant oil	Up to 10% of oil in diet (approx. 132 mg SDA/kg/day)	3 months	No adverse effects reported
Yamazaki *et al.* 1992	Subchronic oral toxicity	12M Wistar rats	SDA ethyl ester	1% of ethyl ester in diet (approx. 1.0 g ester/kg/day)	One or three weeks	No difference in body weight or serum lipid concentrations

Ishihara *et al.* 2002	Subchronic oral toxicity	7M Balb/c mice	SDA triglyceride	10% of TG in diet (approx 2.1 g SDA/kg/day[2])	3 weeks	No differences observed compared to EPA or ALA diets
Crozier *et al.* 1989	Subchronic oral toxicity	7M Dunkin-Hartley guinea pigs	Blackcurrant oil	10% of oil in diet (approx. 104 mg SDA/kg/day)	40 days	No differences observed compared to walnut oil or lard diets
Harris *et al*. 2007	Subchronic oral toxicity	45M Beagle dogs	SDA ethyl esters	Up to 193 mg ester/kg/day in diet (variable percentage)	Up to 12 weeks	No compound-related effects on body weight, food consumption or histopathalogical findings.
Everett *et al*. 1988	Subchronic oral toxicity	75M + 75F Sprague-Dawley rats	Evening primrose oil	Up to 2.5 ml oil/kg/day (approx. 230 mg GLA/kg/day) by gavage	53 weeks	No adverse effects following evaluation of multiple endpoints
Everett *et al*. 1988	Subchronic oral toxicity	15M + 15F beagle dogs	Evening primrose oil	Up to 5.0 ml oil/kg/day (approx. 460 mg GLA/kg/day) by gavage	52 weeks	No adverse effects following evaluation of multiple endpoints
Wainwright *et al*. 2003	Subchronic & reproductive oral toxicity	60F B6D2F_1 mice	Borage oil and GM rapeseed oil	10% of oil in the diet (up to approx. 5.4 g GLA/kg/day)	6 months	No adverse effects attributable to GLA consumption

[1] Numbers treated, excluding controls and animals receiving other treatments. M = Male, F = Female
[2] The authors state the NOAEL > 0.6 g/kg/day SDA but 0.6 g/kg/day was the highest dose tested
[3] The authors state the NOAEL > 1.0 g/kg/day SDA but 1.0 g/kg/day was the highest dose tested

Studies on Buglossoides oil

The safety of Buglossoides oil (supplied by TCI) has been assessed in two unpublished studies in mice (Surette 2013 (in part), Surette and Matar 2012) and one in salmon fry (Plante & Surette 2012).

In the first study, two groups of ten female BALB/c mice (18g) were fed modified Monsanto US17 Rodent Diets supplemented with 0.1g arachidonic ethyl ester/kg of diet. In the treatment group the diet contained Buglossoides oil (26 g/kg diet) so as to provide 1% of the energy as SDA (equivalent to approximately 3.9 g/kg body weight/day of Buglossoides oil and 0.78 g/kg body weight/day of SDA (FDA 1993)). Mice were inspected daily by the animal facility staff for general health status: respiration, colour of paws, muscle tone and signs of distress and dehydration. After three weeks on the diets, 5 animals from each group were given 100 µg/day of biopeptides derived from microbial/enzymatic hydrolysis of dietary proteins by gavage for 7 days, the other 5 animals per group receiving its diluent (saline). The animals were then sacrificed. There were no significant differences in body weight between the dietary groups. Additionally, inspection of the general health status of the animals did not reveal health concerns in any dietary groups.

In the second study, two groups (1 and 2) of 15 female BALB/c mice (18-20g) were fed the control diet and two groups (3 and 4) the diet containing Buglossoides oil (as in the first experiment) for 3 weeks and then groups 1 and 3 were given 100 µg/day of biopeptides by gavage for 7 days, the other two groups receiving its diluent (saline). This was followed by 5 days without treatment and the cycle repeated for the duration of the experiment. After the first 7 days of treatment with biopeptide, mice were then injected with 0.5ml containing 1.4×10^4 4T1 mammary carcinoma cells per ml into the right mammary gland. Tumour volume was measured on days 10, 14, 18, 22 and 27 post-injection. Tumour mass was measured on days of sacrifice; five mice per group were sacrificed on days 12, 20 and 27 post-injection. Mice were inspected daily by the animal facility staff for general health status: respiration, colour of paws, muscle tone and signs of distress and dehydration. There were no significant differences between dietary groups for body weight at day 28 (day of tumour cell injections) and inspection of the general health status of the animals did not reveal health concerns in any dietary group. No significant differences in animal weights between dietary subgroups were observed during the cancer stage of the experiment. The SDA-oil diet showed a trend for decreased tumour growth, with tumour mass being significantly less than the control ($p<0.05$) on days 20 and 27. No safety concerns associated with the dietary regimens were noted.

Plante & Surette studied the effects of Buglossoides oil in Atlantic salmon fry (*Salmo salar* L.) (Plante & Surette 2012). Two isoproteinaceous diets were prepared based on a standard salmonid diet: diet 1 contained 11.5% herring oil and diet 2 was identical except that the herring oil was replaced by Buglossoides oil containing 18.56% SDA. 120 fry weighing on average 1.86g were divided equally amongst six aquaria and fed for four weeks on diet 1, whereupon thirty fish were sampled. The fish in three tanks were then fed for a further 8 weeks on diet 1 whilst the remaining fish were fed for 8 weeks on diet 2. At the end of this period, a further 10 fish per tank (30 per diet) were sampled and fish condition, specific growth rate, mortality, percent lipid deposited (plus fatty acids analysis), and gross energy content were measured. No mortality occurred during the trial in either treatment. After 56 days of feeding, no significant difference was found in terms of growth, fish condition, energy content and specific growth rate between fish fed herring and Buglossoides oil. Small differences in body fatty acid composition

between fish fed the two diets were reported and were ascribed by the authors to the higher content of 18-carbon polyunsaturated fatty acids in Buglossoides oil, and the more elevated content of 20- and 22-carbon monounsaturated fatty acids in herring oil. The content of long chain n-3 fatty acids in the fish bodies was not affected by the type of dietary oil.

Supporting studies

The safety of SDA Soybean Oil was evaluated in a 28-day gavage study and a 90-day one-generation reproduction feeding study in rats (Hammond *et al.* 2008).

In a GLP (Good Laboratory Practice) study that was compliant with FDA, EPA (Environmental Protection Agency) and OECD (Organisation for Economic Co-operation and Development) guidelines, male and female Sprague–Dawley Crl:CD(SD) rats received 20% SDA Soybean Oil at doses of 0, 0.3, 1.0 and 3.0 ml/kg bodyweight by gavage for 28 days. The no-observed-adverse-effect-level (NOAEL) was approximately 3 g/kg/day SDA Soybean Oil, the highest dosage administered. Since the SDA Soybean Oil tested contained 20% SDA, the NOAEL for SDA was approximately 600 mg/kg/day. (The authors state the NOAEL as 'greater than' 600 mg/kg/day SDA but 600 mg/kg/day was the highest dose tested.)

The reproductive and subchronic toxicity of 26% SDA Soybean Oil were evaluated in a GLP-compliant feeding study that was consistent with FDA, EPA and OECD guidelines. Male and female Sprague–Dawley Crl:CD(SD) rats received 26% SDA Soybean Oil as a dietary admixture during a 90-day one generation reproduction feeding period at levels of 0, 1.5 and 4.0 g/kg/day (up to 1000mg SDA/kg body weight/day and 273 mg GLA/kg body weight/day). There were no consistent, dose-dependent, statistically significant adverse effects on any of the parameters tested including clinical observations, haematology, clinical chemistry and urinalysis, organ weights, macroscopic and microscopic pathology and reproductive and developmental endpoints. The NOAEL for reproductive and developmental toxicity and for subchronic toxicity was determined to be 4 g/kg/day, the highest dosage of SDA Soybean Oil tested. Since this SDA Soybean Oil contained 26% SDA, the NOAEL for SDA was 1000 mg/kg/day. (The authors state the NOAEL as 'greater than' 1,000 mg/kg/day SDA but 1,000 mg/kg/day was the highest dose tested.)

A number of other preclinical (safety) studies have been conducted in rats using oils containing SDA. Between 16 and 19 spontaneously hypertensive male rats fed diets containing 11% blackcurrant oil providing approximately 330 mg SDA per kg body weight per day (FDA 1993) for 49 days were not reported to exhibit any compound related adverse effects compared to control animals (Engler 1993). In another study, male Wistar rats received, as dietary admixtures, up to 10% black currant oil (132 mg SDA/kg/day (FDA 1993)) or 10% olive oil (control). The authors reported that the animals appeared healthy and no adverse effects on body, liver, brain or heart weights were observed (Barzanti *et al*. 1995). Yamazaki *et al.* (1992) fed male Wistar rats diets containing 9% lard supplemented with 1% ALA or SDA ethyl esters (approximately 1.0 g ester/kg body weight /day (FDA 1993)) for one or three weeks and reported no differences in body weight or serum lipid concentrations between groups.

Ishihara *et al*. (2002) provided 3-week-old male Balb/c mice with diets containing 1% ALA, SDA or EPA as a triglyceride oil for 3 weeks. There were no reported differences in body weight gain,

food consumption or relative liver weight in the animals. The animals ingested approximately 2108 mg of each of the supplemented fatty acids/kg body weight/day[1].

In male Dunkin-Hartley guinea pigs fed diets containing 10% black currant oil (BCO), walnut oil or lard for 40 days no differences in body weight or liver weights were reported. The BCO diets provided approximately 104 and 684 mg/kg body weight/day of SDA and GLA, respectively (Crozier *et al*. 1989, FDA 1993).

In 15 male Beagle dogs provided diets that contained up to 193 mg of SDA ethyl esters/kg body weight/day for up to 12 weeks, no compound-related effects on body weight or food consumption were reported and liver, heart and kidney appeared normal upon histological examination (Harris *et al*. 2007).

In addition to the above studies with blackcurrant oil, other repeated-dose studies have been conducted with oils containing GLA. For example, in a 53-week study in male and female Sprague-Dawley rats administered evening primrose oil by gavage providing up to 230 mg GLA/kg body weight /day, no adverse effects were reported on any of the multiple endpoints related to safety. A similar study in beagle dogs over 52 weeks providing up to 460 mg GLA/kg/day also reported no adverse effects (Everett *et al.* 1988).

Engler (1993) assessed the effects of dietary GLA from different sources on blood pressure in spontaneously hypertensive rats. 16-19 male rats aged 6-7 weeks were fed diets containing 11% fat which was comprised of either sesame oil (control), evening primrose oil, borage oil, blackcurrant oil or fungal oil for 7 weeks. The diets provided approximately 0, 880, 2090, 1870, 2860 mg GLA/kg body weight/day respectively (FDA 1993). No significant differences in body weight were reported and all GLA treatments were considered to have reduced blood pressure relative to the control. A significant increase in serum cholesterol in the borage oil group was noted after treatment, but no other adverse effects were reported.

Wainwright *et al.* (2003) assessed the effects of dietary GLA from different sources and at different concentrations on reproduction, growth, brain and behavioural development in B6D2F$_1$ mice. Twenty, 8-week old female mice per group were fed diets containing 10% fat which was comprised of either a mixture of 92.7% corn oil and 7.3% soybean oil (control), or borage oil (containing 23% GLA), or genetically modified canola oil (HGCO) containing 23% GLA or HGCO containing 36% GLA for 6 months. The diets provided approximately 0, 3.4, 3.4, and 5.4 g GLA/kg body weight/day respectively (FDA 1993). No significant differences were reported in the number of successful pregnancies, gestation length, or maternal weight gain during gestation and lactation between the groups. Both HGCO groups lost slightly more pups than the control group in the first 2 days after birth. This finding was not considered to be toxicologically significant because of variation in litter sizes and the lack of any other significant reproductive findings.

There were significant differences in the growth of the pups in different groups. The authors stated:

> '*The present findings indicated that some of the effects of GLA provided as HGCO differed from those of equivalent amounts of GLA provided as BO [borage oil].*

[1] Calculated as (Food intake over 3 weeks) / 21 x 1% / (Initial body weight)
(79.7 / 21) x 1% / 0.018 = 2.108 mg/kg/day

Specifically, 23% GLA from HGCO reduced pup body weight and was associated with a slight increase in neonatal pup attrition. There were no significant effects on behavioral development or on performance in the plus maze. An increase in dietary GLA resulted in an increase in brain n-6 FA [fatty acid] and a corresponding decrease in brain n-3. Moreover, despite their similar levels of GLA, the effects on brain FA composition were greater in the mice provided 23% GLA as HGCO than with those receiving the same amount from BO. Comparison of the group receiving 23% GLA from HGCO with that receiving 36% indicated that at the higher level the effects on growth were greater, as were those on brain FA composition, particularly in the PE [ethanolamine phosphoglycerides] fraction. These findings support the use of HGCO as an alternative source of GLA.'

4.III.3.3 Studies in humans

Published studies in humans involving SDA and GLA and ALA are summarised in Table 8.

Table 8 – Summary of toxicology studies in humans

Citation	Objective	Subjects[1]	Test article	Dose	Duration	Results
Harris *et al*. 2008	Oral toxicity	5M + 6F healthy aged 38.4 ± 9.8 years	SDA Enriched Soybean Oil as liquid oil consumed with food	3.66 g/day SDA plus 1.2 g/day GLA	16 weeks	No clinically significant differences reported relative to EPA group or soybean oil control
Lemke *et al*. 2010	Oral toxicity	24M + 30F overweight (BMI 25 to 35 kg/m^2) aged 49.1 ± 12.3 years	SDA Enriched Soybean Oil as liquid oil consumed twice daily with food	4.2 g/day SDA plus 1.13 g/day GLA	12 weeks	No clinically significant differences reported relative to EPA group or soybean oil control
James *et al*. 2003	Oral toxicity	9M + 6 postmenopausal F healthy aged 18 - 65	SDA ethyl ester in capsules	1.5 g/day	6 weeks	No significant change in inflammatory mediators or blood lipids relative to EPA or ALA ethyl ester controls
Surette *et al*. 2004	Oral toxicity	6M + 5F hyper-triglyceridemic aged 56 ± 11 years	Echium oil in capsules consumed thrice daily	1.9 g/day SDA plus 1.7 g/day GLA	28 days	No change in vital signs or clinical laboratory analyses were reported compared to baseline values and no adverse events were attributed to the supplementation
Miles *et al*. 2004a,b and 2006	Oral toxicity	8 M+F healthy aged 33.7 ± 2.6 years	Echium oil in capsules	1 g/day SDA plus 0.9 g/day GLA	12 weeks	No adverse effects on immune response or serum lipids
		12 M+F healthy aged 31.9 ± 2.1 years	Borage oil in capsules	2 g/day GLA		

Kelley *et al*. 1993	Oral toxicity	10 M healthy aged 27.3 ± 1.9 years	Flaxseed oil as liquid with food	20 g/day ALA	56 days	No changes in serum lipids or in coagulation parameters or adverse events
Gracious *et al*. 2010	Oral toxicity	8 M + 7 F bipolar aged 13.4 ± 2.8 years	Flaxseed oil in capsules twice daily	Up to 6.6 g/day ALA	16 weeks	No difference was reported in adverse events or continuation rate in comparison with placebo (olive oil)
Takeuchi *et al*. 2007	Oral toxicity	11 M + 8 F normal or mild hypertensive aged 39.5 ± 13.3 or 36.7 ± 10.9 years	Blended oil as liquid on food	9.1 g/day ALA	4 weeks	No adverse effects were reported relative to control (rapeseed soybean blend)

[1] Numbers treated, excluding controls and subjects receiving other treatments. M = Male, F = Female

Studies involving SDA and GLA have been reported because these fatty acids are not found in traditional foods; studies on ALA because it is the major fatty acid component of Buglossoides oil.

Two clinical studies were conducted using Monsanto's SDA-enriched soybean oil. In the first study (Harris *et al*. 2008), 33 healthy subjects (21 to 70 years old, 14 male, 19 female) were divided into three equal-sized groups and provided with oils in packets and capsules to be consumed with food: either SDA-enriched soybean oil (which provided 3.66g SDA/day and 1.2g GLA/day) in packets with regular soybean oil in capsules; or regular soybean oil in packets and EPA ethyl esters in capsules; or regular soybean oil in both packets and capsules, for 16 weeks. No clinically-significant adverse events were reported from SDA consumption and there were no clinically-significant differences in clinical chemistry parameters compared to subjects consuming 0.98g of EPA ethyl ester/day or regular soybean oil placebo. In the second study (Lemke *et al*. 2010), 252 overweight subjects (aged 49.1 ± 12.3 years) with a Body Mass Index (BMI) of 25 to 35 kg/m^2, consumed SDA-enriched soybean oil providing 4.2g SDA/day and 1.13g GLA/day (24 males plus 30 females), 1g EPA/day (27 males plus 35 females) or regular soybean oil placebo (28 males plus 37 females) for 12 weeks. No significant adverse effects of treatment were reported, including no significant effects on standard clinical chemistry and complete blood count panels, except for two analyses. Total bilirubin was lower in the EPA and SDA groups but remained within the normal clinical range and gamma-glutamyl transpeptidase was lower in the EPA group. The authors stated: "*For both of these differences, no known pathology was associated with the lower values, and the differences were not considered to be important.*"

The consumption of 1.5g/day of SDA ethyl ester in capsules by 9 male and 6 postmenopausal female healthy subjects, aged between 18 and 65, for 6 weeks, resulted in no significant change in inflammatory mediators or blood lipids compared to subjects consuming EPA ethyl esters or ALA ethyl esters (James *et al.* 2003).

Six male and five female hypertriglyceridemic subjects, aged 56 ± 11 years, consumed 15g of echium oil per day containing 1.9g SDA, 1.7g GLA and 4.3g ALA, in capsules three times per day, for 28 days. No change in vital signs or clinical laboratory analyses were reported compared to baseline values and no adverse events were attributed to the supplementation (Surette *et al*. 2004). Serum TG concentrations decreased by 21% during the study, an effect that is well documented following the consumption of dietary omega-3 PUFA from fish oils (Phillipson *et al*. 1985) and which is considered beneficial to health (Tanne *et al*. 2001).

In another study, eight healthy male and female subjects aged 33.7 ± 2.6 years consumed echium oil in capsules which provided 1g SDA/day and 0.9 g GLA /day, and twelve healthy male and female subjects aged 31.9 ± 2.1 years consumed borage oil in capsules which provided 2.0 g/day GLA, both for 12 weeks. No adverse effects on immune response or serum lipids were reported, and no difference in adverse events were reported compared to controls (Miles *et al.* 2004a,b and 2006).

It was reported in 1992 that around 4000 patients had been involved in trials involving the consumption of GLA for more than 3 months, mostly in the form of evening primrose oil. In most of the studies the oil dose was 3-6 g/day providing about 240 – 480 mg GLA/day. In the placebo controlled studies, not a single adverse event occurred more frequently in the GLA group as compared to the control. Evening primrose oil was licensed for use as a pharmaceutical in the United Kingdom and, by 1992, about half a million prescriptions for one month's supply had been

dispensed. The incidence of reported adverse events was far lower than for most drugs and no pattern suggesting a causal relationship to GLA consumption was detected (Horrobin 1992).

Some concern has occasionally been expressed that evening primrose oil (containing 7 – 10% GLA) might cause, or lower the threshold for, epileptic seizures. This was based entirely on two studies reported in the early 1980s. In one study, three patients diagnosed with schizophrenia, who were taking evening primrose oil, developed electroencephalogical features of temporal lobe epilepsy. None of them suffered from a seizure whilst taking evening primrose oil and all three were re-diagnosed during the study as suffering from temporal lobe epilepsy. In the second study, three out of 23 patients with schizophrenia developed seizures, one taking placebo and two taking evening primrose oil. In all cases the patients were also taking phenothiazine-based treatments. There is a strong link between schizophrenia and epilepsy, and it has long been established that phenothiazines lower the threshold for seizures. Puri (2007) therefore concluded that concern about evening primrose oil in relation to epileptic seizures is not warranted.

ALA is one of the two essential fatty acids in humans and is found in many dietary oils including soybean, canola, walnut and flax oils. ALA is the main dietary omega-3 fatty acid in the US diet and its consumption is generally considered to be beneficial to human health. One crossover study of 10 subjects that provided 31g/day of flaxseed oil (20g ALA/day) in the diet for 56 days showed no changes in serum lipids or in coagulation parameters compared to control diets and there were no adverse events reported (Kelley *et al*. 1993). In another study, eight male and seven female bipolar subjects with a mean age of 13.4 ± 2.8 years were provided with flaxseed oil in capsules, twice daily, supplying up to 6.6 g ALA/day. No difference was reported in adverse events or continuation rate in comparison with a placebo group (olive oil) (Gracious *et al*. 2010). In a third study, two groups of subjects (three male and 5 female normotensive aged 39.5 ± 13.3 years and eight male and three female high normal or mild hypertensive aged 36.7 ± 10.9 years) were provided with 7.8 g/day ALA in a blended oil added to food which, in combination with the remainder of their diet provided 9.1 g/day ALA, for four weeks. No adverse effects were observed relative to control groups receiving rapeseed/soybean blended oil (Takeuchi *et al*. 2007)

4.III.4 Other constituents of Buglossoides oil

Erucic acid

Erucic acid is present in Buglossoides oil and is also present in the diet. Diets rich in erucic acid have been reported to cause a transient accumulation of triacylglycerol (lipidosis) in the heart and other tissues of rats (Bremer & Norum 1982). However, this has not been reported in humans. Mustard seed oil (containing 40-60% erucic acid) is the main edible oil in parts of India. In a study comparing post-mortem hearts from one of these areas with those from areas where the main edible oils were peanut, sesame and coconut, fatty acid differences were observed in the heart tissue but it could not be associated with any observed heart damage. In France, where (high-erucic) rapeseed oil is consumed, an epidemiological study in 1974 of 254,788 cases of death due to heart failure found 269 cases (0.11%) with a somewhat similar histology to that seen in rats. The researchers found, in these cases, that there was a significant association with alcohol consumption but not with dietary fat and vegetable oil consumption. These studies and others, reviewed by Food Standards Australia New Zealand (FSANZ 2003) led the authors to conclude that a tolerable level of exposure for humans would be 7.5mg erucic acid/kg bodyweight/day, equivalent to about 500 mg erucic acid/day for the average adult. This would be unlikely to be exceeded if the levels of erucic acid in canola oil (the fully refined, bleached, and deodorised edible oil obtained from certain

varieties of *Brassica napus* or *B. campestris*) did not exceed 2%. Similarly, canola oil is considered GRAS in the USA provided that it contains less than 2% erucic acid (FDA 2012). The level of erucic acid in the fat component of a food product is controlled within the EU to a maximum of 5% of the total level of fatty acids (Anonymous 1976). The levels found in Buglossoides oil are substantially lower than this (see Table 16).

Unsaponifiable fraction

The unsaponifiable fraction of Buglossoides oil has been characterised, as detailed in Table 15. This fraction comprises mainly of phytosterols, principally ß-sitosterol, campesterol and stigmasterol (Table 17), which are all found in major commercial food oils such as corn oil and soybean oil (Gunstone et al. 2007). Phytosterols are considered to be beneficial to human health in that they inhibit the uptake of cholesterol from the diet (Moreau et al. 2002). Buglossoides oil also contains some vitamin E, mostly in the form of γ-tocopherol (Table 18) which is also found at similar levels in a range of common food oils, such as palm oil (Codex 2005).

Pyrrolizidine alkaloids

A number of species from the Boraginaceae family, including *Buglossoides arvensis* (Roeder 1999) contain naturally-produced pyrrolizidine alkaloids (PAs) some of which are liver toxins at high doses (Huizing & Malingré 1981, EFSA 2011). They are carcinogenic in rats although, up to 2001, there was no reported evidence of carcinogenicity in humans (ANZFA 2001). A tentative no-effect level of 10 µg PAs/kg bodyweight/day in the diet has been suggested, based upon limited human data on the incidence of veno-occlusive disease. Applying an uncertainty factor of 10 to this figure allowed the Australia New Zealand Food Authority to propose a provisional tolerable daily dietary intake of 1 µg PAs/kg bodyweight/day (ANZFA 2001).

The European Food Safety Agency (EFSA) has produced a scientific opinion on pyrrolizidine alkaloids in food and feed (EFSA 2011) in which they found that there were no available substantial long-term follow-up data or epidemiological studies to assess whether exposure to 1,2-unsaturated PAs results in cancer in humans. They concluded however that, on the basis of current knowledge of metabolism and other areas, PAs may act as carcinogens and that therefore the data from experimental animals were relevant to humans. On this basis, they calculated the $BMDL_{10}$ (the lower confidence limit on the benchmark dose associated with a 10% response) for the induction of liver haemangiosarcomas by lasiocarpine in male rats at 70 µg/kg body weight/day. This compound is one of the most toxic of the PAs and so represents a conservative choice. Assuming a maximum PA content of 4 ppb in Buglossoides oil and a daily intake at the 97.5th percentile of 103 mg/kg/day SDA (equivalent to 515 mg/kg Buglossoides oil /day), a child would be exposed to 2.6×10^{-3} µg PAs/kg body weight/day, giving a margin of exposure (MOE) of 27,000. The EFSA Scientific Committee has concluded that a MOE of 10,000 or higher, based on a $BMDL_{10}$ from an animal study, is of low concern from a public health point of view.

PAs are present in several foods. Honey made from the nectar of *Echium plantagineum* is believed to make up 10-25% of Australian production (Culvenor 1985 cited in Croda 2006a) and such honey has been shown to contain between 270 and 950 ppb (µg/kg) of PAs. Given usual patterns of honey consumption, it was concluded that this posed little if any human health risk (Coulomb 2003). Refined seed oil from *Echium plantagineum* has been approved as a novel food in the European Union (Anonymous 2008a) and has been notified as a New Dietary Ingredient in the United States without questions being raised by the FDA (Croda 2006b) with a demonstrated upper limit on the concentration of PAs of 4 ppb.

PAs are water-soluble and therefore the bulk of any PAs present in *Buglossoides arvensis* seed would be expected to be left in the seed meal on extraction, and the level in the oil to be further reduced during refining. A sample of unrefined Buglossoides oil has been analyzed and found to contain 44 ppb of PAs, but refining reduces this to below 1 ppb, as indicated in Table 15. At this level, an individual weighing 70 kg would have to consume his own bodyweight of Buglossoides oil each day to reach the tolerable daily intake of PAs set by ANZFA (above), or 0.49 kg/day to reach an MOE of 10,000 as calculated by the EFSA.

Possible allergens

No references in the literature or in the Allergome database have been found of allergic reactions to *Buglossoides arvensis*. Furthermore, the database does not contain any reference to dietary allergens in any members of the Boraginaceae, apart from an unreferenced entry for *Symphytum officinale* (comfrey) which indicates that the leaf contains an unknown allergen (Allergome 2013b). No references to this example have been found in the literature, so it is possible that it is referring to the well known hepatotoxicity of comfrey leaves which is caused by pyrrolizidine alkaloids (EFSA 2011). (The Allergome database does contain two entries for Ehretia species, but these are no longer considered to be members of the Boraginaceae family (Valdés 2004).)

However concerns have been expressed by the ACNFP regarding *Echium plantagineum* which, as has been noted earlier, is closely related to *Buglossoides arvensis* (ACNFP 2007).

Cytochrome c has been reported as a respiratory allergen from echium pollen (Matthews *et al*. 1988) and Sharma *et al.* (2010) consider it to be an important respiratory allergen in the fungus *Curvularia lunata*. It has also been reported as an allergen in grass pollen, but a comprehensive review of grass pollen allergens concluded that "*Taken together, the available evidence indicates that cytochrome c is not a relevant grass pollen allergen and certainly not an important one*." (Andersson & Lidholm 2003). The Allergome database, which contains the allergen data extracted from nearly 6000 scientific papers, does not record any instance of cytochrome c causing an allergic reaction through oral administration (Allergome 2013a). Cytochrome c is an extremely common protein. It is a key enzyme in the mitochondrial respiratory chain and, as such is found in almost all eukaryotic cells, that is to say in all tissues of all multicellular organisms (including mammals, birds, fish, molluscs, insects, plants and algae) and is therefore a component of virtually all foodstuffs which contain protein (Lehninger 1975). Given that no reports of dietary allergy to cytochrome c have been recorded, it is reasonable to conclude that the protein is non-allergenic when ingested.

Respiratory exposure to Buglossoides oil is extremely unlikely as, in common with all vegetable oils, it has an extremely low vapour pressure at room temperature and would decompose or combust in air before reaching its boiling point. The cost and composition of the oil make it unsuitable for high temperature cooking, such as deep fat frying which, in any case, would tend to denature any protein present.

The oil could be atomised to form a mist, and this might occur to some degree if the product was presented in the form of spray container (either pump-action or pressurised) such as the type sold to consumers for applying small amounts of oil for frying or on salads. However, these devices are designed to produce relatively large droplets which do not remain suspended in the air. The protein content of Buglossoides oil is in any case sufficiently low that it would be physically impossible to breathe in sufficient oil to accumulate a meaningful quantity of protein.

TCI therefore believes it is reasonable to conclude that there is no *a priori* reason to suppose that refined Buglossoides oil poses any significant allergenic risk and, as described in section 4.I.1, the level of protein in the oil is sufficiently low that, even if allergens were present, they would not pose an allergenic risk to susceptible individuals.

Other components
A literature survey has been carried out in order to identify possible additional undesirable substances which might be present in the product.

Buglossoides arvensis (and its synonym *Lithospermum arvense*) has not featured widely in the literature, either for reports of its use as food or medicine, or for undesired side effects.

Sandroni (2001), in an historical review of aphrodisiacs found that the leaf and seeds of *L. arvense* had been reported to increase the libido through their androgenic, gonadotropic, and estrogenic properties, but that no toxicity was known. By contrast, Findley & Jacobs (1980) reported that certain Indian tribes in Nevada used a related species (*L. ruderale*) as a contraceptive. They identified antigonadotropic activity in aqueous extracts from the roots.

B. arvensis seeds and leaves were found to give a positive response when treated with appropriate antisera which indicated the presence of phytoecdysteroids (plant-produced analogues of steroidal insect hormones) (Dinan *et al*. 2001). The authors noted that these compounds were apparently non-toxic to mammals and suggested that the ability to synthesis them could be usefully elevated in crop species for the control of insect predators. Phytoectysteroids are found in a wide range of plant species, including food plants. They are highly polar molecules and, if present in cultivated *Buglossoides arvensis* seed, they would be expected to partition into the aqueous fractions during extraction and refining (Soriano *et al*. 2004).

The roots of *Lithospermum erythrorhizon* Siebold & Zucc. have been commonly used in traditional Chinese medicine since at least the 16th century. The active component has been identified as shikonin, a naphthoquinone which has demonstrated wound healing, antitumour and antimicrobial effects in trials. No toxic effects were observed in oral feeding studies in mice or rats, but some toxicity was observed with intraperitoneal administration in mice, giving an LD_{50} of 20 ± 5 mg/kg. Shikonin has not been reported from *Buglossoides arvensis* but has been found in *Echium vulgare* L. Related compounds have been reported from the roots of *Buglossoides arvensis* and various Echium species as shown in Table 9. (Papageorgiou *et al*. 1999)

Weston *et al*. (2012) have reported finding a range of naphthoquinones, including shikonin, acetylshikonin, and 1,3 dihydroxy-3-methylanthraquinone, in roots of *Echium plantagineum*, which they found to provide strong inhibition of plant, insect, fungal, and bacterial growth. Echium oil has been approved for use as a food ingredient in the EU and no concerns have been expressed about the presence of these compounds.

In summary, no reports of significant adverse effects or toxicity in *Buglossoides arvensis* have been discovered in the literature, apart from those which are also associated with Echium oil, which have been discussed above.

Table 9 – Shikonin analogues in *B. arvensis* and *Echium spp.*

	Buglossoides arvensis	Presence in Echium spp.
shikonin	NR	*E. vulgare*
acetylshikonin	Yes	*E. vulgare*
isobutyrylshikonin	Yes	*E. vulgare*
isovalerylshikonin	Yes	*E. vulgare*
isobutyrylshikonin	Yes	*E. vulgare*
a-methylbutyrylshikonin	NR	*E. vulgare*
b,b-dimethylacrylshikonin	NR	*E. vulgare*
b-hydroxyisovalerylshikonin	Yes	*E. lycopsis*
deoxyshikonin,	NR	*E. vulgare*
alkannan	NR	*E. vulgare*

NR = Not reported

4.IX Anticipated intake/extent of use of the novel food

Question: Is there information on the anticipated uses of the novel food based on its properties?

Applicant's answer: Yes

Justification

Buglossoides oil is very similar in its composition to Echium oil with the exception that the proportions of stearidonic and alpha-linolenic acids are higher in Buglossoides oil. Echium oil has been approved as a novel food in the EU (Anonymous 2008a) and a detailed treatment of the anticipated intake was given in the dossier which accompanied the application (Croda 2006a). It is proposed that Buglossoides oil should be used in exactly the same foods and in such proportions as to give the same maximum quantity of stearidonic acid as those approved for Echium oil, as summarised in Table 10 These estimated intakes represent an overestimate of the consumption of SDA from Buglossoides oil, because not all of the food groups used in compiling the estimates were represented in the final approval for Echium oil. Furthermore, it follows from the higher content of SDA in Buglossoides oil that the amount of oil to be used in any food will always be less than the amount of Echium oil which might otherwise have been used.

For instance, to provide the daily maximum quantity of 500mg SDA for a dietary supplement with Echium oil containing 14.7 % SDA (expressed as area % from a commercial sample of oil, as reported in Table 16) would require approximately 3.8 grams of oil:

500 / (0.89 x 0.147) = 3,822

whereas to provide it from Buglossoides oil with 20.3% SDA would require approximately 2.8 grams of oil:

500 / (0.89 x 0.203) = 2,768g

A factor of 0.89 has been used in the above calculations for converting from fatty acid proportion (area %) to proportion of the total oil. This factor allows for the presence of a glycerol moiety in the triglyceride molecule which is not included in the calculation of area %. The factor varies slightly according to the molecular weight of each fatty acid; in the above approximate example it has been calculated on the basis of linoleic acid.

This decrease in oil quantity consumed will substantially offset the increased content of alpha-linolenic acid (ALA) in Buglossoides oil, so that the overall consumption of this fatty acid is similar between the two oils:

Concentration of ALA in commercial Echium oil = 32.6 x 0.89 = 29.0 %

Mean concentration of ALA in Buglossoides oil = 43.8 x 0.89 = 39.0 %

3.8g of Echium oil contains 3.8 x 29.0% = 1.1 g ALA

2.8g of Buglossoides oil contains 2.8 x 39.0% = 1.1 g ALA

Thus, since the SDA and ALA consumption is the same for both oils, so is the omega-3 consumption, as these are the only omega-3 fatty acids present.

All other significant fatty acids are present in Buglossoides oil at a lower proportion than in Echium oil. By a similar calculation to the above, it can therefore be shown that the increased proportion of total polyunsaturates in Buglossoides oil is more than offset by lower oil consumption level, giving an intake of 2.1g of polyunsaturates for Buglossoides oil as against 2.5g for commercial Echium oil.

In summary, in following the SDA intake figures given in Table 10, the intake of all fatty acids (including SDA) from Buglossoides oil and the intake of the oil itself will be lower than has been allowed for in the consumption calculations performed by Croda, which provides an additional margin of safety over and above that provided for Echium oil.

Table 10 – Intended food uses

	Maximum level of stearidonic acid (SDA)	
Use group	**Refined Echium Oil (Anonymous 2008a)**	**Buglossoides oil**
Milk-based products and drinkable yoghurt products delivered in a single dose	250 mg/100 g; 75 mg/100 g for drinks	250 mg/100 g; 75 mg/100 g for drinks
Cheese preparations	750 mg/100 g	750 mg/100 g
Spreadable fat and dressings	750 mg/100 g	750 mg/100 g
Breakfast cereals	625 mg/100 g	625 mg/100 g
Food supplements	500 mg/daily dose as recommended by the manufacturer	500 mg/daily dose 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	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	250 mg/meal replacement

Croda calculated SDA consumption levels for a number of population groups, based on food consumption data collected as part of the UK Food Standards Agency's Dietary Survey Programme (DSP) and on their proposed use levels. Their results are summarised in Table 11 on an intake per person basis and in Table 12 on an intake per kg body weight basis. These results are based on the uses submitted in the novel food application. The following foods were omitted from the list which was approved: dairy analogues, non-carbonated fruit-based drinks, nutrition bars (low-sugar, based on fruit, cereal and/or protein), savoury sauces, bread and bread products. These population use levels are therefore somewhat higher than the levels which would be expected for Echium oil as approved and hence for Buglossoides oil, which has identical proposed uses.

Croda found that usage of their proposed products was high across all population groups with the lowest being 94.3%. Young people (age 4 to 10) had the greatest usage at 99.6%, and the high usage percentages within the groups led to similar all-person and all-user estimates of consumption.

Between the population groups, on a per person basis, male adults were calculated to have the greatest mean and 97.5th percentile intakes of SDA at 1128 and 2175 mg/day, while children had the lowest at 719 and 1351 mg/day. Males had higher intakes than females, and intake increased with age.

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. Female adults had the lowest intakes at 13 and 26 mg/kg body weight. Males still showed higher intakes than females when calculated on this basis, but the calculated intakes decreased with age.

In the highest intake group (male adults), estimated consumption of SDA did not exceed 2200 mg SDA/person/day, equivalent to 11 servings of food at the maximum level of incorporation of Echium oil. Mean consumption was estimated at 1128 mg SDA/person/day, equivalent to 5-6 daily servings. As Croda pointed out, this is a significant overestimate of realistic intakes as it would be extremely unlikely for a person to choose so many products, all with the maximum levels of incorporation of Echium oil. In the case of Buglossoides oil, it is even more unlikely, since there will be a reduced number of categories of product from which consumers may choose, relative to the list on which these estimates are based. Notwithstanding that, the safety studies discussed in section 4.III (in which intakes of up to 4200 mg/person/day were tested) indicate that it is safe to consume SDA at the highest estimated consumption level of 2200 mg/person/day.

These intakes are significantly lower than the intakes anticipated at the mean and 90th percentile for SDA soybean oil, from a genetically modified source which is Generally Recognised as Safe (GRAS) in the USA. This is estimated to supply up to 5.2 g/day at the mean and 10.5 g/day at the 90th percentile (0.09 and 0.20 g/kg body weight/day). (Monsanto 2009a, OFAS 2009b)

Table 11 - Summary of the estimated daily intake of SDA (NDNS data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All Person Consumption				All-User Consumption			
				Mean (mg)	Percentile (mg)			Mean (mg)	Percentile (mg)		
					90	95	97.5		90	95	97.5
Children	1½ to 4½	98.8	1628	719	1053	1216	1354	719	1053	1208	1351
Young People	4 to 10	99.6	834	860	1234	1371	1561	860	1245	1374	1561
Female Teenagers	11 to 18	97.8	436	805	1265	1403	1594	804	1271	1418	1594
Male Teenagers	11 to 18	99.5	414	1056	1647	1873	2076	1057	1647	1873	2091
Female Adults	16 to 64	94.3	903	866	1325	1507	1692	871	1326	1520	1693
Male Adults	16 to 64	95.0	728	1124	1751	1932	2189	1128	1752	1928	2175

From Croda (2006a)

Table 12 - Summary of the estimated per kg daily intake of SDA (NDNS data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All Person Consumption				All-User Consumption			
				Mean (mg)	Percentile (mg)			Mean (mg)	Percentile (mg)		
					90	95	97.5		90	95	97.5
Children	1½ to 4½	98.8	1628	50	76	86	103	51	76	86	103
Young People	4 to 10	99.6	834	34	51	58	68	34	51	58	68
Female Teenagers	11 to 18	97.8	436	15	25	28	32	15	25	28	32
Male Teenagers	11 to 18	99.5	414	20	32	35	39	20	32	35	39
Female Adults	16 to 64	94.3	903	12	20	23	26	13	20	23	26
Male Adults	16 to 64	95.0	728	13	22	24	28	14	22	25	28

From Croda (2006a)

Question: Is there information to show anticipated intakes for groups predicted to be at risk?

Applicant's answer: Yes

Justification

Croda (2006a) identified two groups which they considered might be at risk from eating SDA in Echium oil: those receiving anticoagulant therapy; and those with normal or suboptimal blood triglyceride levels. They concluded that there was no significant risk to either group and did not raise these concerns in their NDI notification to the FDA later the same year (Croda 2006b).

Croda's concern for those receiving anticoagulant therapy was unreferenced, but was based on the conversion of SDA to EPA (although not DHA) as described in section 4.III.3 above. They state that "*. . . the combined intake of EPA and DHA in excess of 3 grams per day has been associated with reductions in platelet aggregation and increases in bleeding time, subjects receiving anticoagulant therapy (e.g., acetylsalicylic acid, warfarin, heparin, etc.) should avoid consuming oils or foods rich in EPA and DHA.*" In the United States, the Food and Drug Administration, has affirmed that menhaden oil is Generally Recognised as Safe (GRAS) provided that the combined intake of EPA and DHA are limited to no more than 3.0 g/person/day (FDA 1997). This limit was imposed because, after a review of the then available evidence, they found that there were no reports of increased bleeding times outside of the normal range at or below that intake level. At intake levels above 3.0 g/person/day they found evidence from a number of trials of increased bleeding time, but found it to be inconclusive because either the extended bleeding times were within the normal range; or, in the case that final bleeding times were beyond the normal range, so also were the initial bleeding times; or the small number of subjects made the data difficult to interpret; or that, in the case of coronary heart disease patients, the changes were not clinically significant; or that the differences were not statistically significant. Because of the lack of data and because of the potential risk of excessive bleeding in some individuals with intakes at higher levels, the FDA concluded that the safety of menhaden oil is generally recognised only at levels that limit intake of EPA and DHA to 3 g/person/day.

More recently, Bays (2007), in a review of the literature, concluded that clinical trials have shown high-dose fish oil omega-3 fatty acid consumption to be safe, even when concurrently administered with other agents that may increase bleeding, such as aspirin and warfarin. Salisbury et al. (2012) reported on a study of 1523 patients across 24 centres who had the levels of red blood cell EPA and DHA (omega-3 index) assessed at the time of their presentation with acute myocardial infarction. The authors had found previously that the omega-3 index was strongly influenced by dietary intake of EPA and DHA in non-fried fish and fish oil supplements. They found no relationship between omega-3 index and bleeding events during hospitalisation, even though the patients were at high risk of bleeding through the use of potent antithrombotic medications and invasive management.

From the above, it is reasonable to conclude that there is no serious risk to health even from intakes of EPA and DHA above 3.0 g/person/day.

In 2012, the European Food Safety Authority concluded that supplemental intakes of EPA and DHA combined at doses up to 5 g/day, and supplemental intakes of EPA alone up to 1.8 g/day, do not raise safety concerns for the adult population (EFSA 2012).

Although there is an enrichment of tissues with EPA following the consumption of oils containing SDA, the efficiency of dietary SDA is inferior to that of dietary EPA itself as shown in both animal and human studies (Hansen-Petrik et al. 2000, Ishihara et al. 2002, James et al. 2003, Harris et al. 2007, Harris et al. 2008, Lemke 2010). In humans, 3 to 5 times more dietary SDA than dietary EPA must be consumed to attain an equivalent enrichment of tissues with EPA (James et al. 2003, Harris et al. 2008). As such, the consumption of 2.2g of SDA per day in Buglossoides oil (representing the 97.5th percentile of the proposed food use in the population group with the highest consumption per person) would fall well below the maximum level of 3g/day for the combined intake of EPA and DHA. Similarly, approximately 10 times more dietary ALA than dietary EPA must be consumed to attain an equivalent enrichment of tissues with EPA (James et al. 2003).

Using the mean fatty acid content of Buglossoides oil as shown in Table 16, it may be calculated that the consumption of 2.2g of SDA from Buglossoides oil containing 20.3% SDA would require

2.2 x 100/20.3 x 1/0.89 = 12.2g of Buglossoides oil.

This amount of oil, with an ALA content of 43.8% would supply

12.2 x 43.8/100 x 0.89 = 4.8 g ALA

Thus the combined production of EPA resulting from this maximal consumption of Buglossoides oil may be calculated as:

2.2 / 3.0 + 4.8/10 = 1.2g EPA

This falls well below the level which is considered to be GRAS when consumed as EPA + DHA and is within the range which EFSA considered to be safe.

The second group identified by Croda who might be at risk were those with normal or sub-normal triglyceride levels. This concern was based on the results of a single study which assessed the effects of Echium oil on 11 subjects with mild to moderate hypertriglyceridaemia (Surette et al. 2004). The authors found that administration of approximately 1.9g/person/day of SDA in the form of 15g Echium oil resulted in a significant reduction in serum triacylglycerol from baseline levels. Croda extrapolated from this that there may be a theoretical risk that individuals with normal or low plasma triglyceride levels may be similarly affected, but concluded that this was not an effect which needed to be taken into account. It seems likely that the triglyceride lowering effect of echium oil is due to the conversion of SDA to EPA in the body, which is confirmed by the fact that fish oil is well known to have a similar effect. TCI has not been able to discover any evidence in the literature that either fish oils or SDA-containing oils lower triglyceride levels in normal and sub-normal individuals. It therefore seems likely that the observed reduction represents a normalisation and would only therefore affect those with high initial triglyceride levels, where it would be a desirable effect.

TCI has not identified any other groups which might be at risk from the consumption of Buglossoides oil. In the absence of any identified at-risk groups, anticipated intake levels for all members of the population are as given under the previous question.

Question: Will introduction of the novel food be restricted geographically?

Applicant's answer: No

Justification

No geographical restrictions in the marketing of Buglossoides oil are anticipated.

Question: Will the novel food replace other foods in the diet?

Applicant's answer: Yes

Justification

It is anticipated that Buglossoides oil will be bought primarily as a replacement for echium oil as it will be cheaper (because the crop is higher yielding) and the higher proportion of SDA means that less oil is needed for the same intake of SDA.

Both oils are more expensive than fish oils so it is thought unlikely that consumption of this source of omega-3 fatty acids will be significantly reduced. It is more likely that the product will be bought by those who wish to increase their intake of long-chain omega-3 fatty acids but don't want to buy fish products either for dietary reasons (e.g. vegetarians) or because they don't like the taste of fish, or because they are concerned about the possible presence of marine pollutants. In this situation, it is likely that the product will replace plant or animal fats which have a lower content of essential fatty acids and, in the case of animal fats, a higher content of saturated fatty acids.

Question: Are any of the replaced foods significant nutritional sources?

Applicant's answer: Yes

Justification

Echium oil is a good source of omega-3 fatty acids, but Buglossoides oil is equivalent at the levels of consumption envisaged.

Both Echium and Buglossoides oils are superior to other current plant-based sources of omega-3 fatty acids such as linseed and hempseed which contain ALA but not SDA. The conversion of ALA to EPA is much less efficient than the conversion of SDA, and so greater quantities of these oils have to be consumed to achieve equivalent EPA production in the body. Therefore, if the market for SDA oils increases beyond its current level, it is likely to be at the expense of these oils which provide lower nutritional value.

Question: Does the probable level of substitution have a nutritional significance for any population group?

Applicant's answer: Yes

Justification

That sector of the population who do not eat fish or fish products (for instance, vegetarians) must fulfil their requirements for EPA largely by endogenous synthesis from dietary omega-3 fatty acids. The only common dietary omega-3 fatty acid is ALA, and as has been described above, approximately 10 times as much ALA must be consumed as EPA in order to achieve the same level of enhancement of tissue EPA. The conversion of SDA to EPA is much more efficient and therefore the consumption of either Echium oil or Buglossoides oil is likely to improve this group's nutritional status. Substitution of Echium oil by Buglossoides oil will not have a significant effect, but the substitution of other plant or animal fats by Buglossoides oil is likely to improve the nutritional status of this group.

4.XI Nutritional information on the novel food

Question: Is there information to show that the novel food is nutritionally equivalent to existing foods that it might replace in the diet?

Applicant's answer: Yes

Justification

As noted above, Buglossoides oil is likely to replace Echium oil or other plant or animal fats. A detailed list of analytical data is given in appendix 1 which allows comparison between a purchased sample of commercial Echium oil and three non-consecutive batches of Buglossoides oil. In section 4.IX, in response to the first question, it was shown that the intake of fatty acids from Buglossoides oil would be the same as or lower than that which is provided by Echium oil. As noted in the answer to the last question of section 4.IX, the replacement of other oils or fats in the diet by Buglossoides oil is likely to lead to an increase in its nutritional value.

4.XII Microbiological information on the novel food

Question: Is the presence of any micro-organisms or their metabolites due to the novelty of the product/process?

Applicant's answer: No

Justification

No micro-organisms are directly involved in the growth of Buglossoides crops or in the production or the oil. Micro-organisms are universally present during field production of crops and so will normally be present on Buglossoides seed. However the process whereby the oil is extracted and

refined (including temperatures in excess of 90°C under vacuum for tens of minutes, and filtration at the micron level) will eliminate most micro-organisms. For comparison, pasteurisation is commonly used for the control of micro-organisms in the production of milk, beer and fruit juices. In the case of milk, EU regulations require that the product be held at a minimum of 71,7°C for 15 seconds (Anonymous 1992).

The oil itself has a very low water content and activity and so does not support subsequent microbial growth. Microbial contamination is not therefore expected to be a significant problem with this product and this is supported by the results of microbiological analyses which are presented in Table 22.

4.XIII Toxicological information on the novel food

As noted below, there is no traditional food which is directly comparable with Buglossoides oil as the significant fatty acids (SDA and GLA) are not found in traditional vegetable oils (although they are present in a number of non-traditional approved vegetable oils). Accordingly, the major part of the toxicological data on the product has been presented in Section 4.III in answer to the question "*Is there information to show that the source organism and/or foods obtained from it are not detrimental to health?*" starting on page 27.

Question: Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment?

Applicant's answer: Yes

Justification

The food which is closest in its characteristics to Buglossoides oil is Echium oil, which was approved for sale as a food in the EU in 2008 (Anonymous 2008a), but this is not a traditional food. There is no traditional food which has similar levels of stearidonic acid and gamma-linolenic acid to Buglossoides oil, but flaxseed oil (the seed oil of *Linum usitatissimum* L., also known as linseed oil) has similar levels of total saturated, omega-3 and omega-6 fatty acids as shown in Table 13.

Table 13 – Comparison between Buglossoides oil and flaxseed oil

	Buglossoides oil[1]	Echium oil[2]	Flaxseed (linseed) oil[3]
Palmitic 16:0	4.5 – 7.5	6.0	6
Stearic 18:0	1.5 – 4.0	3.5	3
Oleic 18:1	4.0 – 10.0	17.2	17
Linoleic 18:2	7.0 – 15.0	18.6	14
GLA 18:3 n-6	3.5 – 8.5	10.2	-
ALA 18:3 n-3	35 – 55	29.5	60
SDA 18:4 n-3	15 – 30	12.6	-
Total omega-3	50 – 85	42.1	60
Total omega-6	10.5 – 23.5	28.8	14
Total saturated	6 – 11.5	9.5	9

[1] From Table 6 [2] Croda (2006a) [3] Gunstone (2005)
All values expressed as percent by weight of total fatty acids.

Question: Compared to the traditional counterpart, does the novel food contain new toxicants or changed levels of existing toxicants?

Applicant's answer: No

Justification

Section 4.III.6 contains a full discussion of those constituents of Buglossoides oil which might pose a risk of toxicity.

Erucic acid is not found in significant quantities in flaxseed oil, but the level found in Buglossoides oil is well below that found in other common food crops and below the applicable regulatory limits.

Pyrrolizidine alkaloids are not found in flaxseed oil but are reduced to below the limits of detection in Buglossoides oil.

Flaxseed proteins can promote an allergic reaction in sensitive individuals. In a prospective study of 1317 patients attending the allergology department at Nancy University Hospital Centre, France, patients were divided into two groups depending on whether they were presenting with food or respiratory allergy (714 individuals) or other, non-atopic symptoms (603 individuals). When challenged with flaxseed proteins using the skin prick test, 12.2% of atopic children and 8.2% of atopic adults were sensitised, whereas none of the non-atopic children and 1% of the adults were

sensitised. Of these, two cases of food allergy to flaxseed were diagnosed and both were adult atopic individuals The authors extrapolated these results to the general population in France, to produce an estimated prevalence of flaxseed sensitisation in the French population ranging from 0.54% to 1.08%. However, they noted that allergy to flaxseed is extremely rare (2 cases out of 1317), corresponding to an estimated 0.01 to 0.02% of the population (1 allergic patient for 6,000 inhabitants) (Fremont *et al.* 2010). Cold-pressed unrefined flaxseed oil is widely available in the EU.

TCI has been unable to discover any reports of allergic reactions to any part of the *Buglossoides arvensis* plant. The refining process has been demonstrated to reduce the level of protein to a sufficiently low level that, even if allergenic proteins were present, they could not provoke a reaction.

Question: Is there information which suggests that the novel food might pose an allergenic risk to humans?

Applicant's answer: No

Justification

See previous question and sections 4.I.1 and 4.III.6.

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5 Evaluation and conclusion by the applicant

Buglossoides oil is a refined vegetable oil extracted from the seeds of *Buglossoides arvensis* (L.) I.M.Johnst., which has not been subject to genetic modification. It is a rich source of omega-3 and omega-6 fatty acids, including the omega-3 fatty acid stearidonic acid (SDA) which is an intermediate in the synthesis of eicosapentaenoic acid (EPA) in the body from dietary alpha-linolenic acid (ALA). SDA is more efficiently converted to EPA than ALA and so dietary sources of SDA are important for individuals who are unwilling or unable to consume EPA directly (for instance from eating oily fish or fish oil supplements). There are other possible significant sources of SDA, but these are either more expensive and less concentrated (in the case of Echium oil) or are derived from a genetically modified organism and are unavailable in the EU (SDA soybean oil).

Conclusion: Buglossoides oil is a potentially valuable new dietary source of omega-3 fatty acids, particularly for those who do not regularly eat oily fish or fish oils supplements.

Buglossoides oil is closely related, taxonomically, and is similar in composition, to Echium oil which is approved for sale as a food in the EU. The fatty acid profiles of the two oils are similar, with the same fatty acids being present; Buglossoides oil having a higher concentration of SDA and ALA and a lower concentration of GLA. The non-saponifiable fractions of the two oils are also similar in composition, comprising largely of sterols and tocopherols.

As a vegetable oil, Buglossoides oil comprises primarily of triglycerides (approximately 90%), with smaller quantities of diglycerides, monoglycerides, glycerol and free fatty acids. These glycerides are broken down during digestion to release the component fatty acids and so the identity of these fatty acids is important. All of the fatty acids in Buglossoides oil are present in other foods which are consumed in the EU in similar or greater proportions.

Conclusion: The presence of all of the identified components of Buglossoides oil in other approved foods provides evidence of safety.

The production process is well defined and controlled, and it has been demonstrated by analysis to result in a stable and reproducible product which meets all applicable EU food requirements. The manufacturing processes are all well characterised and have been widely used in the food industry for many years. No novel processes are used.

Conclusion: The production process does not give cause for concern.

Concern has been expressed about the potential presence of pyrrolizidine alkaloids and allergenic proteins in Echium oil. Since the source of this oil is closely related to *Buglossoides arvensis*, analyses were performed on representative samples of Buglossoides oil which demonstrated that both components, where present, were well below accepted levels. Other inherent constituents which might potentially give rise to toxicity (oxidation products, hydrolysis products, *trans* fatty acids and erucic acid,) were also each considered, analysed and found to be present at well below regulatory limits.

No significant external contaminants have been detected in the oil by analyses for pesticides, elemental contaminants, dioxin and dioxin-like polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), melamine and cyanuric acid.

No micro-organisms are used in the production of the oil. The refining of the oil involves it being held under vacuum at temperatures above 100°C for a period of time which effectively sterilises it, and the oil has a low water content and low water activity so does not support microbial life. This was confirmed by analysis of batch samples, when no microbial contamination was found.

Conclusion: No internal or external toxic components have been identified which will give rise to hazards from the product.

Buglossoides oil is an inherently stable product. The primary route of degradation, as for any polyunsaturated vegetable oil, is through oxidation and, although the oxidation products are known to be harmful, the associated degradation of the taste and smell of the oil is such that it is unlikely that a seriously oxidised oil would be willingly consumed. Care is taken during manufacturing and packaging to exclude air and any of the factors which promote oxidation, and the success of this approach has been confirmed through analysis. As further confirmation, a stability study was undertaken which showed that the oil remained comfortably within specification for peroxide value for a period of eight weeks, even under severely sub-optimal storage conditions.

Conclusion: Buglossoides oil is stable when stored in the absence of oxygen

It is proposed that Buglossoides oil should be used in the same food groups and to an inclusion rate which will give the same level of SDA in the food as has been approved for Echium oil in the EU, with the aim of providing approximately 200mg of SDA per daily serving. This will result in a lower level of inclusion of Buglossoides oil than Echium oil because of the higher SDA content in the former. An assessment of the consumption of Echium oil has been produced, based on the indicated use levels and of food consumption data collected for the UK Food Standards Agency in the Dietary Survey Programme. These estimates represent an overestimate of the consumption of SDA from Buglossoides oil, because not all of the food groups used in compiling the estimates were represented in the final approval for Echium oil. Of the individual population groups assessed, male adults were determined to have the greatest mean and 97.5th percentile all-user intakes of stearidonic acid from echium oil on an absolute basis, at 1128 and 2175 mg SDA/person/day, respectively, while children had the lowest intakes of 719 and 1351 mg SDA/person/day, respectively (Table IX.a-2). Conversely, on a body weight basis, children were identified as having the highest intakes of any population group, with mean and 97.5th percentile intakes of 51 and 103 mg SDA/kg body weight/day respectively.

These intakes are significantly lower than the intakes anticipated at the mean and 90th percentile for SDA soybean oil, from a genetically modified source which is Generally Recognised as Safe (GRAS) in the USA. This is estimated to supply up to 5200 mg SDA/day at the mean and 10500 mg SDA/day at the 90th percentile (90 and 200 mg SDA/kg body weight/day).

Conclusion: The proposed incorporation and consumption rates for oil and SDA are equivalent or lower than those already approved for similar oils.

No groups have been identified which would be at risk through the consumption of Buglossoides oil. It is anticipated that the foods which will be displaced by Buglossoides oil in the EU are either equivalent in nutritional value but more expensive (Echium oil) or are nutritionally inferior (plant sources of ALA). Given this, it is anticipated that the nutritional status of groups who do not eat oily fish or fish oil supplements will be improved.

Conclusion: No negative consequences from the introduction of Buglossoides oil as a novel food have been identified.

The metabolic fate of the oil is well understood and does not give any cause for concern.. The component fatty acids are released from the glycerides upon digestion and are used primarily as an energy source. The essential fatty acids can also be metabolised to longer chain or more unsaturated fatty acids. ALA and SDA can be elongated and desaturated to EPA, the omega-3 fatty acid typically found in fish oils. SDA has not been found to accumulate in human or animal tissues.

Buglossoides oil has been evaluated in three unpublished animal studies and no adverse effects were reported. The principal component fatty acids of the oil have been tested extensively in both animals and humans without any ill effects. Each of the fatty acids present in the oil is also present in foods which are approved for sale in both the USA and Europe. A toxicological comparison with flaxseed oil as a traditional counterpart to Buglossoides oil did not reveal any cause for concern.

Conclusion: The metabolic fate of the oil is well-understood and no adverse effects from the oil or its components have been identified in toxicological trials.

Overall conclusion: On the basis of the information presented in this dossier TCI believes that Buglossoides oil is a safe and wholesome food which will improve the nutritional status of people who do not habitually eat oily fish or fish oil supplements.

Definitions

#	Number
ACNFP	Advisory Committee on Novel Foods and Processes
ALA	alpha-Linolenic acid
AOAC	AOAC International (formerly Association of Analytical Communities)
AOCS	American Oil Chemists Society
AOCS xx xx-xx	Standardised analytical method as published by the American Oil Chemists Society, where xx xxxx is the method reference code.
BCA	Bicinchoninic acid
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
$BMDL_{10}$	Lower confidence limit on the benchmark dose associated with a 10% response
BO	Borage oil
Buglossoides	*Buglossoides arvensis* (L.) I.M.Johnst.
Buglossoides oil	Refined edible oil obtained from the seeds of *Buglossoides arvensis* (L.) I.M.Johnst.
CAS	Chemical Abstracts Services
CBQCA	3-(4-Carboxybenzoyl) quinolone-2-carboxaldehyde
CFR	Code of Federal Regulations
CFU	Colony Forming Units
cGMP	Current Good Manufacturing Practice
CFSAN	Center for Food Safety and Applied Nutrition
CVD	Cardiovascular disease
DGF	German Society for Fat Science (Deutsche Gesellschaft für Fettwissenschaft)
DGLA	dihomo-gamma-linolenic acid
DHA	Docosahexaenoic acid
DL	Dioxin-like polychlorinated biphenyl
DNA	Deoxyribonucleic acid
DSP	Dietary Survey Programme
Echium	*Echium plantagineum* L.
Echium oil	Refined edible oil obtained from the seeds of *Echium plantagineum* L.
Ed	Editor
Eds	Editors
Edn	edition
EEC	European Economic Community
EFSA	European Food Safety Agency
EPA	Eicosapentaenoic acid
EPA	Environmental Protection Agency
et al.	*et alia* (and others)
EU	European Union

F	Female
FA	Fatty acid
FAME	Fatty acid methyl ester
FAO	Food and Agriculture Organisation
FDA	Food and Drug Administration
FDA LIB	FDA Laboratory Information Bulletin
FSANZ	Food Standards Australia New Zealand
GC	Gas chromatography
GC/MS	Gas chromatography followed by mass spectrometry
GLA	gamma-Linolenic acid
GLP	Good Laboratory Practice
GM	Genetically modified
GRAS	Generally Recognised as Safe
GRN	Generally Recognised as Safe Notification
HACCP	Hazard analysis and critical control points
Hg	Mercury
HGCO	Genetically modified canola oil
hr	hour
ICP-MS	Inductively coupled plasma mass spectrometry
ISO	International Organisation for Standardisation
LD_{50}	Median lethal dose
M	Male
MFHPB	Microbiology Food Health Protection Branch (Canada)
MFLP	Microbiology Food Laboratory Procedure (Canada)
µg	microgram (10^{-6} gram)
MOE	Margin of Exposure
n-3	Omega-3 (fatty acid)
n-6	Omega-6 (fatty acid)
n/a	Not applicable
ND	Not detected
NDI	New Dietary Ingredient
NF	National Formulary
NLT	Not less than
NMT	Not more than
NOAEL	No observed adverse effect level
NR	Not reported
NS	Not specified
OECD	Organisation for Economic Co-operation and Development
PA	Pyrrolizidine alkaloid
PAFA	Priority-based assessment of food additives
PAH	Polycyclic aromatic hydrocarbon

PAM	Pesticide Analytical Manual
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzodioxin
PCDF	Polychlorinated dibenzofuran
PE	Ethanolamine phosphoglycerides
ppb	Parts per billion (10^9)
ppm	Parts per million (10^6)
PUFA	Polyunsaturated fatty acid
SDA	Stearidonic acid
spp	Species
TBHQ	tert-butylhydroquinone
TCI	Technology Crops International Inc.
TEQ	Toxic equivalent
TG	Triglyceride
US	United States of America
USA	United States of America
USP	United States Pharmacopoeia
w/w	Mass fraction (weight/weight)
WHO	World Health Organisation

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Appendix 1 – Summary of analytical results

Samples from three non-consecutive batches of manufactured Buglossoides oil were analyzed and the results are shown in Table 14 to Table 22.

Table 14 – Chemical and physical analyses

	Echium oil				Buglossoides oil			
Parameter	**EAL121B**	**EAL121C**	**EAL121D**	**Purchased**	**NZ00053 Batch 4**	**NZ00056 Batch 5**	**NZ00058 Batch 6**	**Analytical Method**
Appearance	Pale yellow liquid	Pale yellow liquid	Pale yellow liquid	Pale yellow liquid	Pale yellow liquid	Pale yellow liquid	Pale yellow liquid	In-house method AP-041
Odour	NR	NR	NR	Slight, characteristic	Slight, characteristic	Slight, characteristi	Slight, characteristi	In-house method AP-005
Colour	NR	NR	NR	1.4R; 12.0Y	0.4R; 4.2Y	0.8R; 8.7Y	0.6R; 7.1Y	AOCS Cc 13j-97
Refractive Index @ 25℃	1.4815	1.4810	1.4805	1.4835	1.4867	1.4840	1.4861	AOCS Cc 7-25
Viscosity @25℃	NR	NR	NR	36.9	42.2	46.4	49.0	Brookfield Instrument Method
Iodine Value	NR	NR	NR	206	233	227	222	AOCS Cd 1b-87
Specific Gravity @ 25℃ (g/ml)	0.9263	0.9279	0.9282	0.931	0.942	.935	.935	AOCS To 1a-64
Flash point (℃)	NR	NR	NR	230	187	176	185	AOCS Cc 9b-55
Cold test	NR	NR	NR	Pass	Pass	Pass	Pass	AOCS Cc 11-53
Peroxide value (meq O_2/kg)	0.28	3.13	1.01	4.64	2.03	1.55	1.22	AOCS Cd 8-53
p-Anisidine value	1.2	5.73	2.5	7.66	12.42	13.13	6.07	AOCS Cd 18-90
Oxidative Stability Index (hr @ 100℃)				0.5	0.30	0.33	0.31	AOCS Cd 12b-92
Acid Value (mg KOH/g)*	0.14	0.16	0.17	0.12	0.22	0.12	0.34	AOCS Ca 5a-40
Moisture (w/w%)	NR	NR	NR	0.05	0.09	0.02	0.07	AOCS Ca 2e-84
Residual solvent (ppm)**	NR	NR	NR	<1.0	n/a***	n/a***	<1.0	In-house GC/MS method

* Acid Value = 1.99 x Free Fatty Acids %. ** Only applicable to solvent-extracted oils *** These batches were cold pressed
NR – Not reported n/a – not applicable GC/MS – Gas chromatography followed by mass spectrometry. Data in columns 2-4 from Croda (2006a)
AOCS – Standardised analytical method as published by the American Oil Chemists Society

Table 15 – Primary constituents

Analyte (%)	EAL121B	EAL121C	EAL121D	Purchased	NZ00053 Batch 4	NZ00056 Batch 5	NZ00058 Batch 6	Method
Triglycerides	NR	NR	NR	93.71	92.58	86.72	89.49	AOCS Cd 11c-93
Diglycerides	NR	NR	NR	2.92	6.11	2.00	2.48	
Monoglycerides	NR	NR	NR	4.13	2.33	3.72	3.93	
Glycerol	NR	NR	NR	<1.00	1.08	<1.0	<1.0	
Epoxy fatty acids	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	AOCS Ce 1h-05
Trans fatty acids	0.77	1.00	0.95	<1.0	<1.0	<1.0	<1.0	AOCS Ce 1h-05
Unsaponifiable matter	0.82	0.80	0.87	0.88	0.28	0.43	0.73	AOCS Ca 6a-40
Sterols (mg/kg)	NR	NR	NR	4770	2560	2160	2430	ISO 12228
Tocopherols & tocotrienols (mg/kg)	NR	NR	NR	965	546	258	390	DGF F-II 4a
Pyrrolizidine alkaloids (µg/kg)	<4 (merged sample)			<1	<1	<1	<1	In-house method
Protein	<10 µg/ml	<10 µg/ml	<10 µg/ml	<10 ppm	<10 ppm	<10 ppm	<10 ppm	Bradford protein assay (Bradford 1976)
Total N (protein/6.25)	NR	NR	NR	NR	<10 ppm	<10 ppm	<10 ppm	Antek 9000NS Analyzer (combustion/ chemiluminescence)
Protein (µg/ml)	NR	NR	NR	1.7	1.3	1.0	1.3	Borate extraction/ CBQCA assay

NR – Not reported. Data in columns 2-4 from Croda (2006a)

Table 16 – Fatty acid composition

	% Composition of total fatty acids (GLC analysis - AOCS Ce 1h-05)								
	Refined Echium Oil (Croda 2006a)				Echium purchased	Buglossoides oil			
	EAL121B	EAL121C	EAL121D	Mean		NZ00053 Batch 4	NZ00056 Batch 5	NZ00058 Batch 6	Mean
Myristic acid (14:0)	NR	NR	NR	NR	<0.1	0.0	0.0	0.0	0.0
Myristoleic acid (14:1)	NR	NR	NR	NR	<0.1	0.0	0.0	0.0	0.0
Palmitic acid (16:0)	6.2	6.0	5.8	6.0	6.6	5.2	5.3	5.2	5.2
Palmitoleic acid (16:1)	NR	NR	NR	NR	<0.1	0.1	0.0	0.1	0.1
Stearic acid (18:0)	3.8	3.5	3.3	3.5	3.3	1.8	1.9	1.8	1.8
Oleic acid (18:1 n-9))	16.9	17.9	16.7	17.2	14.5	7.6	7.6	7.5	7.6
Linoleic acid (18:2 n-6)	19.1	18.9	17.7	18.6	14.6	12.7	12.7	12.7	12.7
alpha-Linolenic acid (18:3 n-3)	29.4	29.3	29.8	29.5	32.6	44.0	44.0	43.5	43.8
gamma-linolenic acid (18:3 n-6)	10.5	9.6	10.6	10.2	11.6	6.4	6.2	6.3	6.3
Stearidonic acid (18:4 n-3)	12.5	12.5	12.7	12.6	14.7	20.5	19.7	20.8	20.3

Arachidic acid (20:0)	Trace	Trace	1.3	Trace - 1.3	<0.1	0.0	0.0	0.0	0.0
Gondoic acid (20:1 n-9)	0.8	0.8	0.8	0.8	0.7	0.7	0.8	0.8	0.8
Dihomolinoleic acid (20:2 n-6)	NR	NR	NR	NR	<0.1	0.0	0.0	0.0	0.0
Dihomolinolenic acid (20:3 n-3)	NR	NR	NR	NR	<0.1	0.0	0.0	0.0	0.0
Arachidonic acid (20:4 n-6)	NR	NR	NR	NR	<0.1	0.0	0.0	0.0	0.0
Behenic acid (22:0)	<0.3	<0.3	0.3	<0.3	<0.1	0.0	0.0	0.0	0.0
Erucic acid (22:1 n-9)	0.3	0.4	0.7	0.5	0.3	0.2	0.2	0.2	0.2
Lignoceric acid (24:0)	NR	NR	NR	NR	<0.1	<0.1	<0.1	<0.1	<0.1
Nervonic acid (24:1)	NR	NR	NR	NR	0.2	0.1	0.0	0.0	0.0
(n-3)% total	41.9	41.8	42.5	42.1	47.3	64.5	63.7	64.3	64.2
(n-3) + (n-6) % total	71.5	70.3	70.8	70.2	73.5	83.6	82.6	83.3	83.2

Method: AOCS Ch 2a-94, Ce 1f-96
NR = Not recorded
Values expressed as % of total fatty acids

Table 17 – Phytosterol content

Analyte (% sterols)	EAL121B	EAL121C	EAL121D	Purchased	NZ00053 Batch 4	NZ00056 Batch 5	NZ00058 Batch 6	Canola Oil (CODEX 2005)	Vegetable Oil Phytosterols* (Cargill 2000)	Arabica Coffee (Valdenebro *et al*. 1999)
Cholesterol	NR	NR	NR	0.2	0.7	0.6	0.7	ND – 1.3	0.36	1.2
Campesterol	27.9	23.5	26.3	30.4	37.1	39.4	38.7	5.0 – 13.0	23.58	15.4
Campestanol	NR	NR	NR	0.3	<0.1	2.3	1.8	NR	0.89	0.73
Stigmasterol	NR	NR	NR	1.1	0.4	0.4	0.5	0.2 – 1.0	23.24	18.9
Δ-7-Campesterol	NR	NR	NR	4.0	0.8	0.6	0.5	NR	0.71	0.6
Chlerosterol	NR	NR	NR	0.9	0.4	0.3	0.4	NR	NR	0.87
β-Sitosterol	18.6	12.0	18.5	26.9	47.2	43.1	42.8	45.1 – 57.9	42.27	52.7
Sitostanol	NR	NR	NR	0.4	0.5	0.7	0.7	NR	NR	2.41
Δ-5-avenasterol	18.0	9.3	14.1	18.3	7.3	5.5	7.8	2.5 – 6.6	0.82	2.84
Δ-5,24-Stigmasterol	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.6
Δ-7-Stigmastenol	NR	NR	NR	0.4	0.3	0.2	0.2	ND – 1.3	0.72	2.04
Δ-7-Avenasterol	NR	NR	NR	2.6	1.4	1.2	1.2	ND – 0.8	0.26	1.74

REFINED BUGLOSSOIDES OIL

Analyte (% sterols)	EAL121B	EAL121C	EAL121D	Purchased	NZ00053 Batch 4	NZ00056 Batch 5	NZ00058 Batch 6	Canola Oil (CODEX 2005)	Vegetable Oil Phytosterols* (Cargill 2000)	Arabica Coffee (Valdenebro *et al*. 1999)
24-Methylene-cholesterol	5.3	13.1	4.2	9.0	2.2	1.7	2.6	NR	NR	NR
Brassicasterol	NR	NR	NR	<0.1	<0.1	<0.1	<0.1	5.0 – 13.0	0.45	NR
** Δ 5,23 stigmastadienol	NR	NR	NR	1.8	0.2	2.7	1.1	NR	NR	NR
Δ 5,24 stigmastadienol	NR	NR	NR	3.5	1.5	1.3	1.0	NR	NR	0.6
Others	30	42.1	36.9	<0.1	<0.1	<0.1	<0.1	ND – 4.2	3.46	NR

Method: ISO 12228
ND = Not Detected.
* Mean results from five samples. This material has been notified as GRAS without objection (OFAS 2000).
** Δ 5,23 stigmastadienol is present in olive oil and is one of the six phytosterols which are required to form at least 93% of the sterol content under the International Olive Oil Council trade standard. (Vossen 2007).
Data in columns 2-4 from Croda (2006a)

Table 18 – Tocopherol and tocotrienol content

Analyte (mg/kg)	Echium oil EAL121B, C & D	Purchased Echium oil	Codex soybean oil specification*	NZ00053 Batch 4	NZ00056 Batch 5	NZ00058 Batch 6
α-tocopherol	Not recorded	105	9 – 352	<10	<10	<10
β-tocopherol	Not recorded	<10	ND – 36	<10	<10	<10
γ-tocopherol	Not recorded	719	89 – 2307	535	258	390
δ-tocopherol	Not recorded	141	154 – 932	11	<10	<10
α-tocotrienol	Not recorded	<10	ND – 69	<10	<10	<10
β- tocotrienol	Not recorded	<10	Not specified	<10	<10	<10
γ- tocotrienol	Not recorded	<10	ND – 103	<10	<10	<10
δ- tocotrienol	Not recorded	<10	ND	<10	<10	<10
Total	Not recorded	965	600 – 3370	546	258	390

* CODEX 2005
ND = Not detected
Method: DGF FII-4a

Table 19 – Potential external contaminants

Analyte	EU Limit*	EAL121B	EAL121C	EAL121D	NZ00053 Batch 4	NZ0056 Batch 5	NZ0058 Batch 6	Method
Arsenic (mg/kg)	-	<0.10	<0.10	<0.10	<0.007	<0.007	<0.007	ICP-MS/ AOAC 993.14
Cadmium (mg/kg)	1.0[FS]	<0.01	<0.01	<0.01	<0.002	<0.002	<0.002	
Copper (mg/kg)	-	<0.1	<0.1	<0.1	<0.013	<0.013	<0.014	
Iron (mg/kg)	-	<0.1	<0.1	<0.1	<0.95	<0.94	1.16	
Lead (mg/kg)	0.1	<0.10	<0.10	<0.10	<0.007	<0.007	<0.007	
Mercury (mg/kg)	0.1[FS]	<0.005	<0.005	<0.005	<0.004	0.010	0.004	
Nickel (mg/kg)	-	<0.1	<0.1	<0.1	0.018	0.013	0.020	
Silver (mg/kg)	-	NR	NR	NR	<0.07	<0.07	<0.07	
Tin (mg/kg)	200[CF]	<0.2	<0.2	<0.2	<0.03	<0.03	<0.03	
Total heavy metals as lead (mg/kg)	-	<10	<10	<10	<10	<10	<10	USP/NF 231
Pesticides	Various	ND	ND	ND	ND	ND	ND	FDA PAM 304 E3C5
Melamine (mg/kg)	2.5 total	NR	NR	NR	<0.05	<0.05	<0.05	FDA LIB 4422
Cyanuric acid (mg/kg)		NR	NR	NR	<0.25	<0.25	<0.25	

* Commission Regulation (EC) No 1881/2006 (Anonymous 2006) NR – Not recorded
[FS] Food supplements only [CF] Canned foods only ND – Not detected Data in columns 3-5 from Croda (2006a)

Table 20 – Dioxins and dioxin-like PCBs

Analyte	EU Limit	EAL121B	EAL121C	EAL121D	NZ00053 Batch 4	NZ00056 Batch 5	NZ00058 Batch 6
PCDD/PCDF - WHO TEQ with DLs (pg/g) (Sum of dioxins)	0.75[1]	0.331	0.156	0.258	0.36	0.21	0.21
WHO TEQ with DLs (pg/g) (Sum of dioxin-like PCBs)		0.105	0.0608	0.0595	0.171	0.100	0.0990
Sum PCDD/PCDF and /Dioxin-like PCBs –WHO TEQ with DLs (pg/g)	1.5[1]	0.436	0.217	0.318	0.531	0.31	0.309

[1] Commission Regulation (EC) No 1881/2006 (Anonymous 2006)
In-house method. Data in columns 3-5 from Croda (2006a)

Table 21 – Polycyclic aromatic hydrocarbons (PAHs)

Analyte (µg/kg)	EU Limit*	NZ00053 Batch 4	NZ00056 Batch 5	NZ00058 Batch 6
acenaphthene		<1.0	<1.0	<1.0
acenaphthylene		<2.0	<2.0	<2.0
anthracene		<3.0	<3.0	<3.0
Benzo[a]anthracene		<2.0	<2.0	<2.0
benzo[a]pyrene	2.0	<2.0	<2.0	<2.0
benzo[b]fluoranthene		<3.0	<3.0	<3.0
Benzo[ghi]perylene		<3.0	<3.0	<3.0
benzo[k]fluoranthene		<4.0	<4.0	<4.0
chrysene		<1.0	<1.0	<1.0
dibenz[a,h]anthracene		<3.0	<3.0	<3.0
fluoranthene		<1.0	<1.0	<1.0
fluorene		<2.0	<2.0	<2.0
indeno[1,2,3-cd]pyrene		<3.0	<3.0	<3.0
naphthalene		<2.0	<2.0	<2.0
phenanthrene		<2.0	<2.0	2.3
pyrene		<1.0	<1.0	<1.0
Sum of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene	10	ND (<8.0)**	ND (<8.0)**	ND (<8.0)**

* Commission Regulation (EC) No 1881/2006 (Anonymous 2006)
** Sum of detection limits
In-house method QA049

Table 22 – Microbiological tests

Test (cfu/g)	EAL121B	EAL121C	EAL121D	Purchased	NZ00053 Batch 4	NZ0056 Batch 5	NZ00058 Batch 6
Total aerobic plate count	NR	NR	NR	<5	<5	<5	<5
Osmophilic yeast	<10	<10	<10	<5	<5	<5	<5
Yeasts	<10	<10	<10	<5	<5	<5	<5
Moulds	<10	<10	<10				
Enterobacteria	<10	<10	<10	ND	ND	ND	ND
Staphylococcus aureus	<10	<10	<10	ND	ND	ND	ND

ND = Not detected
Methods Used USP <61>, MFLP 43, MFHPB-22
Data in columns 2-4 from Croda (2006a)